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CHEMOTROPISM OF FUNGI.[1]

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HISTORICAL.

DE BARY (**5**) advanced the supposition that the oogonia of certain Phycomycetes not only attract the antheridia-producing branches, but determine the formation of these branches as well. The same writer (**6**) later raises the question as to whether the bending of a germ-tube toward the epidermis of its proper host, but not toward every membrane or moist surface, may not be brought about by a specific reaction in the parasite, induced by physical or chemical stimuli which may be supposed to operate through unknown secretions from the host plant. This was written, though not published, before PFEFFER'S first studies (**25**) on the reactions of motile unicellular organisms to chemical stimuli. During the progress of his studies with Saprolegnia swarm-spores, he observed that the hyphae of the fungus turned toward the nutrient substances, and he supposed that chemical agents might in many cases determine the direction of growth of these and other hyphae.

KIHLMAN (**16**) observed that if a germinating ascospore of *Melanospora parasitica* lies at a distance of not more than four or five spore-diameters from a growing hypha of its host, *Isaria farinosa*, the direction of growth of the latter is deflected toward the spore of the parasite until there is contact with its germ-tube.

According to BREFELD (**1**), the fact that neighboring sporidia of Ustilaginaceae conjugate in pairs, the connecting tubes taking the shortest course between two sporidia, may be explained by assuming a directive chemical influence.

[1] Contribution No. 4 from the Botanical Laboratory of the University of Missouri.

WORTMANN (**36**) observed the turning of young germ-tubes of *Saprolegnia* sp., and concluded that these are very sensitive to chemical stimuli, especially to those concerned in nutrition; a most energetic turning was observed toward flies' legs.

MARSHALL WARD (**32**) mentions two factors as mainly influencing the direction of growth of "lily-botrytis," namely, the contact of hyphae with one another or with solid bodies, and the direction in which food lies in relation to the growing hyphae.

WORONIN (**34**) holds that it is through chemical influence that the conidial germ-tubes of *Peziza baccarum* reach the wounds of the host plant.

STRANGE (**29**) made careful observations on growing hyphae of *Saprolegnia ferax* for the purpose of confirming their reported turning toward nutrient substances. That the hyphae turn toward a region of diffusing nutriment was regarded as very questionable; there was noticed, however, a stronger growth of the hyphae in the nutrient region, and the hyphae, by their branching, became more abundant here than elsewhere. The germinating conidia sent their tubes in all directions, provided enough nutriment for growth was present, and not markedly toward any area containing a greater amount of nutriment. Similar results were obtained with germ-tubes from Penicillium spores; these showed no chemotropic turning, but a much better growth when they reached the diffusion region around the openings of the capillary tubes containing the test solutions.

REINHARDT (**28**) found that the direction of growth in *Peziza* sp. is affected by chemical influences. Gelatin containing sugar and spores of *Mucor* sp. have an attractive effect. Spores of *Aspergillus niger*, *A. flavus*, and *Penicillium glaucum*, as well as colonies of various bacteria, cause a cessation of growth, which is followed by a reversal of the direction of growth.

BÜSGEN (**2**) observed chemotropism in the case of *Botrytis cinerea*, and supposed that a chemical stimulus might cause the germ-tubes in the dew-drop to seek the host epidermis; but he considered that penetration is brought about by contact influence. He asserts the possibility of chemotropism in the case of germ-tubes from uredo-

spores and from the conidia of Peronosporaceae, and of chemotaxis in the case of Cystopus swarm-spores.

Many of the foregoing are merely opinions or passing observations, made in the course of investigation of other phenomena. MIYOSHI (**20**), however, made chemotropism of certain fungi the subject of systematic and extensive experimental study. The principal fungi used by him were *Mucor stolonifer*, *M. mucedo*, *Phycomyces nitens*, *Penicillium glaucum*, *Aspergillus niger*, and *Saprolegnia ferax*. The tests were made with the aid of perforated membranes, such as strips of epidermis, celloidin films, and mica plates; of capillary tubes; and of injected leaves and petioles of *Tradescantia* sp. He concludes that, in the case of the fungi enumerated, molecules of many substances diffusing from the openings cause diversion of the hyphae from their original direction of growth, the turning being either toward the diffusing substance (positive chemotropism), when the substance is attractive to the fungus, or away from the substance (negative chemotropism), when the substance is repellent. Some substances are wholly or almost wholly neutral. The direction and amount of turning are dependent upon the concentration. Chemotropism is most marked at an optimum concentration, which varies for the substance and the fungus. The concentration just sufficient to cause turning is very low for most attractive substances. Repellent substances are acids, alkalis, alcohol, certain neutral salts and toxic compounds; also very strong solutions of substances that are neutral or attractive at lower concentrations. Generally attractive substances are ammonium nitrate, ammonium chlorid, ammonium malate, ammonium tartrate, potassium phosphate, sodium phosphate, ammonium phosphate, meat extract, peptone, sugar, asparagin, etc. For chemotropic phenomena Weber's law holds. The effect of an attractive substance may be overcome by the presence, in sufficient quantity, of a repellent substance.

The same investigator (**21**), in connection with his study of the penetration of natural and artificial membranes by fungi, found that the hyphae of *Botrytis cinerea* and *Penicillium glaucum* would grow through a membrane only when they were placed on nutrient substrata; there would be, however, no penetration through the membrane to

the substratum if the fungi were grown in a nutrient medium, although the mycelial growth was more vigorous.

SWINGLE (**31**), in explanation of the effects of Bordeaux mixture, advanced the hypothesis, which he based on the studies of REINHARDT, BÜSGEN, and MIYOSHI, that copper hydroxid may prevent the germ-tubes of parasitic fungi from entering the host plant through negative chemotropic action.

NORDHAUSEN (**24**) accepted MIYOSHI'S conclusions, and investigated the biological bearing of chemotropism upon the penetration of plant tissues by certain fungi, without bringing forward any additional evidence in favor of chemotropism.

No further investigation seems to have been made with relation to fungi, until CLARK (**4**), in his investigation of SWINGLE'S hypothesis, had occasion to test the chemotropic reactions of certain species, especially *Mucor stolonifer*, to toxic substances. For the most part he followed MIYOSHI'S methods closely. In every case it was found that the hyphae would turn from a nutrient medium and grow into media containing such toxic substances as salts of copper, cobalt, nickel, zinc, etc., until a concentration sufficient to cause death was reached. The hyphae turned toward non-nutrient media and distilled water as readily as toward nutrient media. His conclusion is that *Mucor stolonifer* is negatively chemotropic to some secretion of its own mycelium, and that this negative chemotropism is much greater than any positive chemotropism it may have for food substances or oxygen.

MASSEE (**19**) found that fungi are attracted to their hosts by specific stimuli from substances in the cell sap. In the case of saprophytes and facultative parasites, the attractive substance is saccharose; the facultative parasites, however, may be repelled by more potent negatively chemotropic substances in the cell sap. In the case of obligate parasites, the cell sap of the host plant is the strongest positive chemotropic agent; malic acid is the specific attractive substance for the germ tubes of *Monilia fructigena*, and the enzyme pectase for *Cercospora cucumis*. Immune plants owe their immunity to the absence of the chemotropic substance.

Other factors affecting the direction of growth of fungous hyphae have received but little attention, while the causes of the bending

of sporangiophores, especially of certain Mucorineae, have been carefully studied.

A negative hydrotropism for the sporangiophores of *Phycomyces nitens* was first experimentally established by WORTMANN (**35**), and was later confirmed by the more extended studies of ERRERA (**10**) and of STEYER (**30**). MOLISCH (**22**) showed that the sporangiophores of *Mucor stolonifer* and *Coprinus velaris* are negatively hydrotropic; while KLEBS (**17**) made similar observations for *Sporodinia grandis*, which FALCK (**12**) has failed to confirm.

WORTMANN (**35**) observed what he supposed to be negative hydrotropism in the case of the mycelium of *Mucor* sp., which would grow towards water, but would turn aside and branch profusely on reaching its surface. The conditions of experiment were such as to make his explanation of the phenomenon doubtful. STEYER (**30**) concludes that moisture plays an unimportant rôle in determining the growth and spreading of the mycelium of *Phycomyces nitens*.

JÖNSSON (**15**) grew mycelia of *Mucor stolonifer*, *Phycomyces nitens*, and *Botrytis cinerea* on sloping filter-paper strips having their two ends dipped in vessels of water at different levels. Phycomyces and Mucor showed negative rheotropism under these conditions, while Botrytis showed positive rheotropism.

HOFMEISTER (**14**), WORTMANN (**35**), DIETZ (**7**), KLEBS (**17**), and STEYER (**30**) have established for various Mucoraceae a negative reaction of their sporangiophores to gravity and to strong light; but there is a positive reaction to contact and to moderate intensities of light.

KNY (**18**) holds that gravity has no effect upon the growth of the mycelium of *Mucor mucedo*, *M. stolonifer*, *Trichothecium roseum*, and *Eurotium repens*. MIYOSHI (**20**) concludes from his tests that neither gravity, light, contact, nor moisture affected the turning of the six fungi used in his investigations. STEYER (**20**) in his study of the reactions of Phycomyces, found that the mycelium is indifferent to light, contact, and gravity.

MATERIALS AND GENERAL METHODS.

To a greater or less extent fourteen species of fungi have been used; these, with respect to nutritive adaptations, may be classed as follows:

STRICT PARASITES	STRICT SAPROPHYTES
Uromyces caryophyllinus	Mucor stolonifer
FACULTATIVE SAPROPHYTES	Mucor mucedo
Sphaeropsis malorum	Phycomyces nitens
Cercospora apii	Penicillium glaucum
Monilia fructigena	Monilia sitophila
FACULTATIVE PARASITES	Sterigmatocystis nigra
Botrytis vulgaris	Coprinus micaceus
Daedalia quercina	Agaricus fabaceus

With exceptions as noted below, spores of the various fungi from pure cultures one to two weeks old were used in making inoculations. *Sphaeropsis malorum* was not ready in pure culture as soon as needed, and inoculations for the first experiments with this form were made directly from infected apple twigs; the spores were found to germinate quickly and the hyphae grew rapidly, so that the observed bacterial and mold contamination in these cultures was slight. *Cercospora apii*, obtained from celery leaves, was grown in artificial culture on pieces of sugar beet; spores were not produced, but satisfactory inoculations were made with small portions of detached mycelium. Inoculations in the case of the three Hymenomycetes were made with portions of mycelium from pure cultures, which had been made from sporophores by the "tissue-culture" method (DUGGAR, **9**). Spores of *Uromyces caryophyllinus*, taken directly from carnation leaves, were used to some extent. The germination of these was not certain under all conditions, and the growth was limited; the use of the fungus was soon abandoned. All other spores gave perfectly satisfactory germination in gelatin and agar-agar media. Even such species as *Penicillium glaucum* and *Sterigmatocystis nigra*, which have been found (DUGGAR, **8**) not to germinate in distilled water, gave a germination of practically 100 per cent. in gelatin and agar made up with distilled water.

Precautions were taken to have all apparatus chemically clean and thoroughly sterile. Glassware and mica plates were boiled in alkali and in acid, and again, after a thorough rinsing, in distilled water. Covers were rinsed in 95 per cent. alcohol, then wiped with a sterile cloth. Heavier glassware and mica plates were sterilized with dry heat at a temperature of 140° to 150° C. Celloidin films were sterilized by being boiled in redistilled water; strips of epidermis, by being

rinsed in alcohol and afterwards soaked in two changes of sterile redistilled water. The media used were sterilized, whenever possible, under 15 lbs. pressure of steam, or by fractional sterilization at 100° C. Except in those experiments in which capillary tubes were used, contaminations were of rare occurrence; whenever contamination was apparent, the experiment was disregarded in tabulating results. With few exceptions the chemicals used were the chemically pure preparations of reliable manufacturers. The water used in making up test solutions was redistilled in glass apparatus. All experiments were made in duplicate, and were repeated when occasion demanded. A fairly constant temperature of 28–29° C. was maintained for the cultures. Beet decoction, made by boiling 450^{gm} fresh weight of sugar beet root in 1,000^{cc} tap water, was the usual basis of nutrient agar and gelatin media. The stock decoction was diluted two to ten times for use.

TESTS FOR CHEMOTROPISM.

The capillary tube method.—In the first tests PFEFFER'S (**26**) method with capillary tubes was used. These tubes were filled with the chemical solutions under the air-pump, were rapidly rinsed in sterile distilled water, and were placed on the cover glasses in drops of the culture medium, while it was still liquid. The cover glasses were then inverted over Van Tieghem cells made up in the way described by CLARK (**3**), and were sealed to the cell rim with vaseline. A small quantity of liquid, the same in composition as that used in making up the culture medium, was placed in the bottom of each cell. Observations were made when the hyphae were 40–75 spore-diameters in length, and again three or four hours later when growth had become indefinitely great. In estimating the effects of the chemicals, regard was had for the hyphae from spores lying within a radius of one lumen diameter from the opening of the tube, and such other hyphae from more distant spores as entered this area. Only those were held to be chemotropically affected that showed a turning out of their former courses toward or away from the tube opening. In recording the observations MIYOSHI'S method was used; to denote a turning away of 12 per cent. to 37 per cent., the symbol *r* was used; for a turning away of less than 12 per cent. and an attraction of less than 12 per cent., the symbol *o;* for an attraction

of 12 per cent. to 37 per cent., a_1; for an attraction of 37 per cent. to 62 per cent., a_2; etc. An interrogation point indicates a value near the lower limit of the class indicated.

This method was used in the preliminary testing of a large number of representative chemical substances with *Sterigmatocystis nigra* and *Mucor mucedo; Botrytis vulgaris* was used with a few that gave decided effects. The series included the sulfate of sodium, of magnesium, of calcium, of ammonium, of potassium; the normal phosphate of ammonium, of potassium; the dibasic phosphate of ammonium, of potassium; the monobasic phosphate of potassium, of sodium, of calcium; the chlorid of potassium, of ammonium, of calcium, of magnesium, of sodium, of lithium; the nitrate of potassium, of ammonium, of calcium, of magnesium, of sodium; the oxalate of magnesium, of potassium; the tartrate of magnesium, of potassium, of sodium-potassium; the bitartrate of potassium; the lactate of magnesium; the malate of magnesium, of ammonium; the acetate of potassium; acetic, lactic, tartaric, malic, oxalic acids; cane sugar, dextrose, galactose, maltose, lactose, starch; glycerin, ethyl alcohol; active pepsin, boiled pepsin, trypsin, peptone, mannit agaricin, casein, asparagin, urea; meat extract, beet decoction, bean decoction, distilled water; mercuric chlorid, copper sulfate, copper acetate, lead nitrate, zinc nitrate, iron sulfate, iron chlorid, potassium ferrocyanid. In the case of such of these compounds as were tested by MIYOSHI, concentrations were used that would give the maximum effect of attraction or repulsion. With nearly all compounds two, and with many three, concentrations were tested.

The hyphae showed a tendency, in small numbers, to turn toward the tubes in the majority of tests; but in only a few instances were more than about 37 per cent. of the hyphae in the observed area

TABLE I

SPECIES	$CaH_4(PO_4)_2$, 2%	KH_2PO_4, 2%	$MgSO_4$, 2%	$NaNO_3$, 2%	$(NH_4)_2HPO_4$, 2%	NH_4NO_3, 2%	Acetic Acid, 5%	Malic Acid, 5%	Pepsin (active), 2%	Pepsin (boiled), 2%	Trypsin, 1%	Meat Extract, 10%	Ammonium Malate, 2%
Sterigmatocystis nigra	r	...	r	...	...	r	...	...	...	...	...	...	r
Mucor mucedo	...	a_2	...	r	a_2	...	r	a_2	a_2	a_2	a_2	a_2	a_2
Botrytis vulgaris	...	...	...	...	...	...	...	...	a_2	a_2	...	...	...

affected. The repellent effect was much less marked, never affecting more than one-fourth of the hyphae, and even in the most marked cases some of the hyphae would grow toward the tubes and enter them. Table I gives the most marked instances of attraction and repulsion noted in the series; the symbols have been explained above.

In Table II is given a comparison of the effects of a number of representative compounds as observed by MIYOSHI and in the present investigation. Leaving out of consideration *Saprolegnia ferax*, the extreme effects recorded in MIYOSHI's table were obtained

TABLE II

REAGENT	MUCOR MUCEDO		ASPERGILLUS NIGER	STERIGMATOCYSTIS NIGRA
	I	II	III	IV
Monobasic potassium phosphate, 2 %	a_3	a_2	a_1	a_1
Monobasic sodium phosphate, 2 %	a_2	a_2 ?	a_1	a_1
Neutral ammonium phosphate, 2 %	a_4	a_1	a_2	a_1
Neutral ammonium phosphate, 0.2 %	...	a_1	...	a_1
Neutral ammonium phosphate, 0.1 %	a_1	...	a_1	...
Ammonium nitrate, 2 %	a_3	a_2 ?	a_2	r
Potassium nitrate, 2 %	r	a_1	r	o
Calcium nitrate, 2 %	r	a_1	r	o
Magnesium sulfate, 2 %	r	o	r	r
Ammonium chlorid, 3 %	a_3	o	a_1	o
Potassium chlorid, 2 %	r	o	r	o
Sodium chlorid, 2 %	r	o	r	o
Sodium-potassium tartrate, 2 %	r	a_2 ?	r	o
Cane sugar, 20 %	a_1	o	a_1	o
" " 10 %	a_4	...	a_3	...
" " 5 %	...	o	...	o
" " 1 %	a_3	a_1	a_2	a_1 ?
" " 0.1 %	a_1	o	a_1	o
Lactose, 2 %	a_1	...	a_1 ?	...
" 1 %	...	a_1	...	a_1 ?
Maltose, 2 %	a_1	...	o	...
" 1 %	...	o	...	a_1 ?
Dextrose, 10 %	a_4	...	a_2	...
" 5 %	...	o	...	a_1 ?
" 2 %	a_4	...	a_3	...
" 1 %	a_3	r ?	a_1	o
Ethyl alcohol, 1 %	r	a_2 ?	r	a_2 ?
Glycerin, 10 %	a_1	o	a_1 ?	a_1 ?
Asparagin, 2 %	a_2	a_2 ?	a_1	a_1 ?
Urea, 2 %	o	a_1 ?	o	a_1 ?
Peptone, 2 %	...	o	...	a_1 ?
" 1 %	a_3	...	a_2	...
Meat extract, 10 %	a_4	a_2	a_4	...
" " 1 %	a_3	a_1	a_2	a_1
" " 0.1 %	a_1	o	a_1	a_1 ?
" " 0.01 %	a_1	o	o	a_1 ?

with *Mucor mucedo* and *Aspergillus niger;* the values given by him for these forms are to be found in columns I and III below. It was impracticable to use *Asperillgus niger* in the present study, and *Sterigmatocystis nigra* was substituted with the understanding that the two forms are not always distinguished. The present results for *Mucor mucedo* and *Sterigmatocystis nigra* are given below in columns II and IV respectively.

In the control cultures, where distilled water was used in the tubes, the effect was the same as for the majority of chemical substances; that is, 10–30 per cent. of the hyphae turned toward the tubes. The same amount of positive turning was observed in the case of all the strongly toxic compounds used. Even with a 0.05 per cent. solution of mercuric chlorid and a 1 per cent. solution of copper sulfate, which completely inhibited germination within a radius of eight to twelve tube diameters from the openings, the hyphae not only grew across the diffusion areas, but 10–30 per cent. of those approaching the openings turned toward them and grew for a considerable distance into the tubes.

Although four concentrations of cane sugar ranging from 20 to 0.1 per cent. and four concentrations of meat extract ranging from 10–0.01 per cent. were used, no definite relation between the strength of stimulus and that of response was apparent.

Two corresponding series were made with ten representative compounds; in one series sugar-beet agar was the culture medium; in the other, distilled-water agar. No difference in the behavior of the hyphae due to a difference in the media in which they grew could be observed.

Tests with mica plates.—Thin sheets of mica were cut into pieces about 25×16^{mm}; these were perforated with a needle, the holes being $0.1\text{–}0.15^{mm}$ in diameter, and about 2^{mm} apart. Covers of suitable size were cut from glass 1^{mm} thick. A layer of gelatin or agar was placed on a cover, a mica plate was placed on this just before it hardened, and a second layer was placed above the plate. The chemical to be tested was made up in double strength solution in redistilled water, and one volume of this was added to one volume of gelatin or agar, also made up double strength in redistilled water. It was usually found convenient to have the layer containing the

chemical next to the cover, the spores being distributed in the outer layer. The cover was inverted over a stender dish of 36mm diameter containing distilled water, and was sealed with a coating of vaseline around the rim of the vessel.

This method seemed to possess distinct advantages over the one with capillary tubes: the numerous perforations made it possible to make a large number of observations from a single preparation;

TABLE III

	I. Monilia fructigena	II. Monilia sitophila	III. Sterigmatocystis nigra	IV. Botrytis vulgaris	V. Cercospora apii	VI. Sphaeropsis malorum
Neutral potassium phosphate, 2 %	12	19	24	22	33	22
" " " 0.2 %	21	20	18	25	36	25
Monobasic potassium phosphate, 2 %	29	22	24	26	36	23
" " " 0.2 %	20	26	23	20	25	21
Dibasic ammonium phosphate, 2 %	13	20	21	20	15	23
Calcium phosphate, 0.2 %	49	41	34	31	35	29
Phosphoric acid, 0.2 %	48	30	35	39	23	30
Malic acid, 1 %	×	×	24	×	38	×
" " 0.1 %	21	21	26	21	17	×
Tartaric acid, 1%	×	20	43	22	19	18
" " 0.1%	25	27	34	25	24	22
Oxalic acid, 1 %	×	17	23	×	17	×
" " 0.1 %	23	22	23	34	11	40
Cane sugar, 5 %	38	32	24	31	20	48
" " 0.5 %	30	37	20	30	20	55
" " 0.05 %	30	35	24	33	23	42
Glucose, 5 %	39	39	20	34	30	37
" 0.5 %	40	24	23	39	20	30
Peptone, 2 %	14	28	21	13	20	19
" 0.2 %	26	23	20	21	13	22
Pepsin (active), 2 %	24	20	19	42	23	30
" " 0.2 %	25	30	22	27	31	22
Pepsin (boiled), 2 %	26	31	36	33	40	37
Asparagin, 0.1 %	19	23	30	23	30	15
" 0.1 %	15	23	30	20	30	24
Copper sulfate, 0.005 %	29	21	21	25	34	31
Dibasic sodium phosphate, 2 %	35	25	26	26	15	20
" " " 0.2 %	38	30	27	20	26	23
Starch paste, 0.4 %	34	24	20	21	27	25
" " 0.4 %	44	27	22	20	26	15
Maltose, 2 %	33	30	22	21	25	14
" 0.2 %	36	29	28	31	30	28
Control (distilled-water gelatin)	28	30	29	24	22	18

NOTE: The symbol × indicates that germination was inhibited. *Uromyces caryophyllinus* was tested with the majority of these substances, in the few instances in which growth was sufficiently great for a determination, the turning was practically the same as for the other fungi.

the medium containing the chemical and that containing the spores could be more nearly equalized in amount and in consistency; fewer hyphae would take a course that would lead them through the openings without apparent turning; better sterilization could be secured; and there was less difficulty in making up the preparations.

In making the counts, hyphae within a radius of one opening diameter from the margin of each opening were considered; the hyphae within such an area were classed in the counts as those turning toward the openings, those turning away from the openings, and those apparently indifferent. After an examination of the entire preparation in each case, those holes were selected for the counts which represented the average condition. In calculating the percentages from the counts, the difference between those attracted and those repelled was made the dividend, and the total number within the observed area was made the divisor. The results are shown in Table III.

If the percentages of turning toward distilled-water gelatin as determined by the control experiments, be deducted from the percentages of turning toward the various chemical compounds, it will be found that in only three instances would the difference, which would be supposed to indicate the amount of turning due to chemical influences, approximate 25 per cent., or about the average of the lowest of the several degrees of positive chemotropism recognized by MIYOSHI. Most of the values, even for supposedly highly attractive substances, are very near the controls.

Evidently, the results thus far have not been favorable to the theory of chemotropism. But it was thought that the fungi, all of which grew perfectly normally in gelatin made up in distilled water, might turn more strongly from some other medium in which there was less of available nutriment, to one having an abundance. In agar made up with distilled water, the fungi germinated after a greater length of time and grew more slowly; but the turning from this non-nutrient agar toward a nutrient salt solution favorable for fungous growth was no more marked than from agar containing the same proportions of the nutrient salts to the nutrient salt solution; nor did the nutrient solution seem to attract from either medium more strongly than did distilled water. This test was made with

Sterigmatocystis nigra, *Mucor mucedo*, and *Mucor stolonifer* by the capillary tube method.

It is possible, although there is evidence against it in some of the previous experiments, that the diffusion of the solutes might be rapid enough to bring about practical uniformity in the media before the germination of the spores. To determine whether the time at which the stimulus is applied has an influence upon the reaction, series were made up with two of the more slowly growing species, *Botrytis vulgaris* and *Monilia fructigena*, the spores being distributed in non-nutrient gelatin placed above the mica plates. Four duplicate series were arranged. In one the layers of gelatin containing the substances to be tested were placed below the plates at the time of making up the cultures; in another series these layers were added just as the spores began to germinate; in a third, after the most vigorous hyphae had attained a length of 15 to 20 spore-diameters; in a fourth when the length equaled 40 to 50 spore-diameters. The final counts were made a little later, when the growth was about 75 spore-diameters. The results are given in Table IV for the five compounds tested and the control. The numbers in columns I indicate the average number of hyphae in the area around each hole; those in columns II, the percentages of turning toward the holes.

TABLE IV

Species	Hours Until Application	Dextrose 4.5%		$CuSO_4$ 0.01%		Oxalic acid 0.05%		KH_2PO_4 2%		$(NH_4)NO_3$ 2%		Non-nutrient gelatin	
		I	II	I	II	I	II	I	II	I	II	I	II
Botrytis vulgaris	0	11	35	11	38	6.3	34	4.0	37	5.5	36	9.2	34
	10	9.0	35	6.0	30	7.8	36	5.4	30	7.2	36	7.6	34
	13	11	33	5.5	34	6.4	25	6.4	31	11	33	7.2	35
	16	3.3	30	7.6	34	5.5	30	5.8	35	5.0	32	5.6	36
Monilia fructigena	0	9.3	38	10	30	11	28	3.7	31	8.2	34	4.4	26
	10	9.5	36	11	30	11	30	3.7	28	6.8	25	11	32
	15	13	33	7.5	27	10	32	4.3	30	5.4	27	13	27
	20	17	32	8.7	27	9.3	29	5.3	29	5.1	26	8.3	30

It is evident that the time of application has little or no effect upon the amount of turning. It will also be seen by comparing these

results with former ones, that it is immaterial, as far as the percentage of turning is concerned, whether the spore-containing layer is above or below the one containing the test substance.

Other culture media were used, such as 10 per cent. and 25 per cent. glycerin, gelatin made up to contain 10 per cent. of glycerin and to contain 5 per cent. of cane sugar. With none of these were there more distinct indications of chemotropism than in former tests, in which non-nutrient gelatin was the culture medium. This would indicate that the available nutriment and the concentration of the medium have no effect.

The final test along this line was made with silica jelly, a medium free from organic matter and of suitable consistency. The method of preparation was that used by MOORE (**23**), except that dialysis was accomplished satisfactorily with parchment paper. In order to secure the proper coagulation of the medium, it was necessary to add mineral salts to all media used. A nutrient salt solution, containing 1^{gm} of ammonium nitrate, 0.5^{gm} of monobasic potassium phosphate, 0.25^{gm} of magnesium sulfate, a trace of ferric chlorid, and 5^{gm} of glucose, in a volume of 100^{cc}, was made the basis of the work. It would seem that if fungi show chemotropism under any conditions it would be by turning from a medium lacking some one or more of the elements necessary for full development, toward the diffusion centers of compounds supplying the missing element or elements, or toward a full nutrient solution.

In the tests each compound in turn, except ferric chlorid, was omitted from the silica jelly containing the spores, and in each case jelly containing the omitted compound in proper proportion on the one hand, and full nutrient jelly on the other were used on the opposite side of the mica plate from the above-mentioned spore-containing layer. Control tests were carried on with jelly lacking one and the same compound on each side of the plate, and also with full nutrient jelly on each side of the plate. To reduce evaporation, the lower layer was covered with an unperforated mica plate. The results are given in Table V, in percentage of response by turning from the first-named medium in which the spores were sown to the contrasted one.

There is a very striking uniformity in the percentages, and this

TABLE V

Contrasted Cultures in Silica Jelly	Mucor stolonifer	Sterigmatocystis nigra	Monilia sitophila	Botrytis vulgaris	Monilia fructigena
Full nutrient : full nutrient	35	32	32	33	29
Lacking glucose : lacking glucose	32	36	37	30	33
Lacking glucose : containing glucose	27	30	34	30	26
Lacking glucose : full nutrient	30	36	33	30	30
Lacking KH_2PO_4 : lacking KH_2PO_4	33	30	29	33	26
Lacking KH_2PO_4 : containing KH_2PO_4	36	36	31	30	28
Lacking KH_2PO_4 : full nutrient	32	30	33	36	28
Lacking $(NH_4)NO_3$: lacking $(NH_4)NO_3$	35	35	33	36	28
Lacking $(NH_4)NO_3$: containing $(NH_4)NO_3$	34	32	32	33	29
Lacking $(NH_4)NO_3$: full nutrient	34	33	33	31	32
Lacking $MgSO_4$: lacking $MgSO_4$	33	31	33	33	27
Lacking $MgSO_4$: containing $MgSO_4$	39	34	33	33	29
Lacking $MgSO_4$: full nutrient	29	33	32	35	30

under conditions that would be presumed to be most favorable for chemotropic reaction.

Tests with epidermis and celloidin films.—To test the effect of physically different perforated sheets as well as effectually to repeat the methods used by former investigators, use was made of celloidin films which had been perforated, and of strips of epidermis of *Yucca aloifolia*. This gave, with reference to physical properties, a range from the wholly impermeable mica plates on the one hand to the semipermeable celloidin films on the other.

The tests with epidermis were made with *Monilia fructigena*, *Sterigmatocystis nigra*, *Botrytis vulgaris*, *Sphaeropsis malorum*, and *Mucor stolonifer*, the spores of which were distributed in non-nutrient 8 per cent. gelatin above the epidermis in its final position; gelatin layers containing 5 per cent. cane sugar, 4.5 per cent. dextrose, 0.01 per cent. copper sulfate, 0.1 per cent. oxalic acid, 0.2 per cent. phosphoric acid, and non-nutrient gelatin, were spread below the epidermis. Under these conditions the penetration of the stomates or turning toward the stomates was practically zero. When no culture medium was used, the spores being merely spread on the under surface of the epidermis, hyphal growth was good. A few hyphae of each species grew through the stomates; but there was no evident turning toward them, and in no case was there penetration of more than one or two per cent. of the stomates.

In similar series with celloidin films, the turning from one gelatin layer to another was about equal to that obtained with mica plates. When the spores were spread on the film without a culture medium, very few of the hyphae grew through the holes, the percentage of turning being negligible. The hyphae in these cases were surrounded by a distinct film of condensed moisture.

TESTS FOR OTHER FACTORS.

The tests thus far have failed to give evidence of the existence of any marked chemotropism. There has been at the same time a considerable and fairly constant turning of the hyphae from a medium containing spores to a sterile medium, when these were separated the one from the other by any one of several partitions. Since this turning is apparently unaffected by the chemical relationships of the media employed, the cause of the turning must be sought in other factors. Two possibilities at first present themselves; the mechanical partitions may have a thigmotropic or other influence, or the germinating spores themselves may affect the direction of growth.

Tests without mechanical partitions.—A slight modification of the method employed by CLARK (4) was used. A large drop of agar, 8^{mm} in diameter, was placed in the center of a sufficiently large square of glass; this drop was surrounded by four drops of about 5^{mm} diameter, equidistant from the first, and with a space of about 3^{mm} between each smaller drop and the larger one. Non-nutrient agar and 10 per cent. sugar-beet agar were used for the drops, and were arranged in four combinations: the central drop was of nutrient agar and two small drops diagonally opposite each other were of nutrient agar, the other two being non-nutrient; the central drop was of nutrient agar, two small drops adjacent to each other of nutrient agar, and the other two of non-nutrient agar; two similarly arranged combinations had central drops of non-nutrient agar. The fungi used were *Monilia sitophila*, *Mucor stolonifer*, and *Botrytis vulgaris*. A few spores were sown with the platinum needle at the center of the large drop in each preparation; the cover was inverted over a stender dish containing distilled water and was sealed to its rim. The growth was watched until the hyphae had grown about two-thirds of the distance from the center to the margin

of a large drop; the preparations were then opened, and with a sterile needle the small drops were pushed up until their edges came in contact with the larger drops. Later observations showed that the hyphae of the three fungi grew readily from either medium into a similar or a dissimilar medium, and with the same percentage of turning. An equal amount of turning toward the agar drops was observed in the case of those hyphae which had grown beyond the bounds of the larger drops on the moist glass; whether the agar was nutrient or non-nutrient seemed immaterial. The turning was apparent at a considerable distance from the surface film in so large a percentage of cases as to negative the supposition that the physical condition of the film has an influence.

A further test without mechanical separation was made by placing small crystals of cane sugar, copper sulfate, oxalic acid, monobasic potassium phosphate, and ammonium nitrate in the center of layers of non-nutrient gelatin on cover glasses. Spores of *Monilia fructigena, Botrytis vulgaris, Sterigmatocystis nigra, Mucor stolonifer,* and *Monilia sitophila* were used; in some instances they were evenly distributed in the gelatin, in other instances the gelatin was inoculated by being touched with the needle at several points varying in distance from the crystal. In no case was there any distinct turning toward or away from the diffusion center.

The effect of hyphae upon the direction of growth.—CLARK (4) explains his results by supposing that the fungus secretes some substance to which the growing hyphae are negatively chemotropic. While this hypothesis would very well explain his results, he seems not to have made it the subject of experimental study.

It may be reasonably assumed that if a fungus is negatively chemotropic to its own secretion, the stimulus to turn away from an area containing the fungus would, in early stages of growth, be in some degree proportional to the amount of mycelium in that area.

To determine whether the amount of mycelium has an effect, inoculations were made with differing numbers of spores; the growth was from non-nutrient gelatin and gelatin containing M/4 solution of dextrose, and was toward similar as well as different media as indicated in Table VI, where the results are given. The direction of growth is from the first-named medium to the second. Columns

I give the average number of spores per hole, and columns II give the percentages of turning toward the holes.

TABLE VI

Contrasted-Cultures in Gelatin	Sterigmatocystis nigra		Monilia sitophila		Botrytis vulgaris		Monilia fructigena		Mucor stolonifer	
	I	II	I	II	I	II	I	II	I	II
Non-nutrient : M/4 dextrose.....	4.1	34	5.0	24	6.8	27	5.0	20	15	31
	15	32	16	25	22	37	7.3	26	54	36
M/4 dextrose : M/4 dextrose.....	8.5	24	4.2	25	8.3	29	4.8	26	5.0	25
	22	36	23	38	23	37	21	36	20	38
M/4 dextrose : non-nutrient.....	8.0	25	7.7	26	6.7	28	6.0	28	6.3	31
	19	35	17	36	23	37	18	29	14	31
Non-nutrient : non-nutrient.....	3.0	33	5.0	30	8.0	31	4.5	22	7.1	28
	21	40	16	40	18	39	17	34	36	41
M/4 glycerin : M/4 dextrose.....	3.9	30	4.3	27	6.3	20	7.0	28	17	29
	22	38	19	39	18	36	18	34	31	35
Non-nutrient : 0.01% $CuSO_4$....	3.1	24	5.3	28	6.3	28	5.0	27	18	33
	20	39	15	39	21	39	16	32	43	39

There is indication that the number of hyphae in a given area and the amount of turning from that area are correlated. It may be said of this, as of succeeding tests, that it is at best only relative. It is manifestly impossible to eliminate growing hyphae from the experiments, and their effect is the very factor to be tested.

A test was made by comparing preparations in which only one layer of gelatin was inoculated, with preparations in which both layers were inoculated. Two parallel series were prepared: in one the spores in the lower layer were about twice as numerous as in the other series. An examination of Table VII, which gives the results, shows that the percentage of turning toward a layer containing hyphae is less than toward a sterile layer; and in either case the abundance of mycelium in the layer influences directly the turning

TABLE VII

Monilia sitophila			Sterigmatocystis nigra			Mucor stolonifer			Mucor mucedo			Phycomyces nitens		
I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
6.5	0	26	6.5	0	32	5.5	0	32	11	0	29	7	0	28
12	0	35	15	0	38	14	0	39	23	0	34	14	0	37
4.5	7	20	5.0	8	23	4.5	7	15	11	19	25	9	16	24
9	6	22	12	8	24	9.5	7	24	19	11	30	16	9	29

of the hyphae from that layer. Column I gives the average number of hyphae per hole in the layer *from* which the turning takes place; column II, the average number of hyphae per hole in the layer *toward* which turning takes place; column III, the percentage of turning.

To determine whether this negative turning is due largely to chemical changes in the culture medium, beet decoction in which a fungus had grown was tested with that fungus. The sugar-beet decoction was diluted to one-third the strength of the stock solution. When a good amount of mycelium had been formed, the liquid was filtered under sterile conditions at room temperature. The filtrate was replaced in the incubator in the case of those species which had fruited, and was allowed to remain for twenty-four hours in order that any spores that had passed through the filter might germinate; it was then refiltered. In this way a practically sterile medium was obtained. One volume of this decoction was added to one volume of double-strength gelatin, and tests were made with this

TABLE VIII

	A		B	
	Growth toward Gelatin Made Up with Beet Decoction in Which Fungus Had Grown		Growth toward Gelatin Made Up with Fresh Beet Decoction. Control	
	I	II	I	II
I. Botrytis vulgaris	6.2	29	5.3	33
	15	31	15	35
II. Sterigmatocystis nigra	10	29	9.0	34
	19	27	17	37
III. Penicillium glaucum	8.0	23	12	31
	18	22	28	23
IV. Monilia sitophila	8.0	22	9.0	26
	23	25	20	33
V. Mucor stolonifer	19	32	22	38
VI. Agaricus fabaceus	49	24	50	32
VII. Coprinus micaceus	31	24	43	30
VIII. Daedalia quercina	46	26	35	31

in comparison with control beet decoction in which there had been no fungous growth. The results are shown in Table VIII. Column I gives the average number of hyphae per hole; column II, the percentage of turning toward the holes.

The percentages for all of the fungi in this table, except *Mucor*

stolonifer, have been computed from four distinct series. In this way erratic results have been nullified, and the difference in effect, although not marked, may be regarded as constant. There is certainly a lessened attractive influence in the case of the decoction in which the fungi have grown; this might be due to the mere abstraction from the decoction of nutrient substances, or to the conversion of compounds occurring in the decoction into compounds which are repellent in their effect, or to the secretion of products by the fungus which have a repellent effect. That the first is probably not the case is to be inferred from previous tests, in which these fungi have been found to grow as readily toward distilled water and other non-nutrients as toward nutrients; a mere decrease, therefore, in the amount of available nutriment could hardly have a pronounced effect. We must conclude, therefore, that a medium in which a fungus has grown may become less attractive, or more repellent, to the fungus through the agency of some undetermined substance or substances, which are secreted or otherwise formed by the growing fungus; this reaction would be a special kind of negative chemotropism.

Miss FERGUSON (**13**) found that germinating spores of *Agaricus campestris*, or bits of older growing mycelia, have a very marked effect in causing the germination of spores of this species; at the same time there seems to be a retarding effect upon the growth of the protruded germ-mycelium. Mycelium that is not growing, masses of ungerminated spores, or growing mycelia of other fungi do not have the same influence over germination. Her observations lead her to suppose that oxygen or carbon dioxid is not the determining factor, but that some secretion is formed which stimulates or makes possible the emission of the germ-tubes. Other observations relative to the influence of germinating spores upon the growth of fungi have been made by KIHLMAN (**16**) and REINHARDT (**28**); to these reference has already been made.

Numerous instances have been recorded of the influence by various plant cells upon the direction of movement and of growth of other cells of the same or a different kind, and the general terms cytotaxis and cytotropism have been applied to this peculiar sort of chemical influence. In the cases enumerated by PFEFFER (**27**,

sec. 155), the effects seem to be due either to the excretion of a hypothetical specific substance which furnishes the stimulus, or to changes in the relative proportions of oxygen and carbon dioxid through respiratory or photosynthetic activities. Since these phenomena seem to be analogous in a general way to the above-described turning of fungous hyphae, and since the term cytotropism indicates nothing as to the exact nature of the ultimate stimulus-substance, this term might be found a convenient one for designating the present cases.

The effect of moisture.—The circumstance that a number of preparations in which the culture medium had become evidently dry, gave large percentages of turning, suggested that the hyphae might react to a hydrotropic stimulus. Layers of agar containing spores of several fungi were spread on cover glasses, and sterile strips of filter paper were placed in contact with the agar and allowed to dip into the water of the Van Tieghem cell. The average percentage of turning toward the strips for those spores within a distance of 0.35^{mm} from their edges was 40 per cent. for *Penicillium glaucum;* 50 per cent. for *Mucor stolonifer, Monilia sitophila,* and *Sterigmatocystis nigra;* 55 per cent. for *Mucor mucedo;* 57 per cent. for *Botrytis vulgaris;* and 60 per cent. for *Monilia fructigena* and *Sphaeropsis malorum.*

The percentages given are less than they would be if a smaller area about each strip had been considered; this may be due in part to the circumstance that the spores were rather thickly sown, and the hyphae from those nearest the strip, being numerous, exerted an effective repellent influence on those more distant, causing them to grow away from the strip. Notwithstanding this, the evidence of positive hydrotropism for these fungi was quite conclusive.

As a further test, mica plates were cut to fit Van Tieghem cells and were perforated; a drop of non-nutrient gelatin was placed on each and covered with a perforated plate small enough to fit inside the cell; upon this was placed another layer of non-nutrient jelly containing the spores; another perforated plate was added, and a third layer of gelatin, sterile like the first; the lower surface of this was left uncovered. The mica cover was inverted over a cell containing water, during the time required for the proper growth of the fungi, evaporation took place from the now uppermost layer to

the surrounding atmosphere, as was apparent from the dry condition of the gelatin around the holes in the uppermost plate; water diffused from the lower layers to supply the deficiency. In this way it came about that the middle layer, which contained the spores, was moister than the uppermost layer, but drier than the lowermost. There was observed a very decided turning of the hyphae toward and through the openings in the third plate, which separated the middle and lowermost layers, while comparatively few grew toward the uppermost layer. The estimated percentages are shown in Table IX.

TABLE IX

Directions	Monilia fructigena	Monilia sitophila	Sterigmatocystis nigra	Botrytis vulgaris	Penicillium glaucum	Sphaeropsis malorum	Mucor mucedo	Mucor stolonifer	Phycomyces nitens
From middle layer to lowermost layer..	43	70	80	55	68	65	73	63	65
From middle layer to uppermost layer..	20	20	20	30	18	15	18	15	18

Experiments were set up in which very firm gelatin (16 per cent.) containing the spores was covered with mica plates having a few perforations. The plates were sealed to the covers by an application of vaseline around their margins. The covers were then inverted over stender dishes level full of sterile distilled water. In this way the water came in contact with the gelatin only through the perforations, and diffused from these through the gelatin layer. Hyphae of *Mucor stolonifer* grown under these conditions showed a tendency to grow toward the openings from a distance of 1.5^{mm}, but on coming within 0.5^{mm} of the openings, the course was changed, and the hyphae circled the openings in lines more or less concentric with their margins. The majority of those nearer the openings than 0.5^{mm} grew in a radial direction away from them. In a few instances hyphae grew into the water. *Mucor mucedo* showed a quite decided turning toward the holes, and about 65 per cent of the hyphae within a radius of three hole-diameters turned through them and grew into the water. With *Botrytis vulgaris* about 40 per cent., and with *Penicillium glaucum* 85 per cent. of the hyphae within a corresponding area were affected positively. In every case the growth

in the water was in all directions, directly downward, as well as radially in a horizontal plane. The value of the control cultures, which were duplicates in all respects excepting that the dishes were only partly filled with water, was vitiated by the accumulation of condensed moisture in comparatively large drops about the openings in the plates. This caused an unmistakable turning toward the holes, which was not so decided, however, as in the test cultures.

It is evident from these results that all of the fungi tested in this regard are, under the conditions of experiment, positively hydrotropic; but *Mucor stolonifer* may under certain conditions show a negative hydrotropism. This response to a hydrotropic stimulus probably accounts in large measure for the constant turning toward protected layers from those more exposed, which latter may have become drier through evaporation.

A sharp distinction between hydrotropism and rheotropism on the one hand, or between hydrotropism and osmotropism on the other, cannot in all cases be made, although these phenomena in typical cases are quite distinct. The phenomena here reported are probably due primarily to differences in the moisture content of the layers, and not to water currents, either molar or molecular. For this reason the term hydrotropism has been applied, which is not in agreement, however, with the current view (PFEFFER, **27**, p. 592), that in the case of the fungous mycelia heretofore studied, osmotropism and rheotropism, but not hydrotropism, have been established. It is further recognized that chemical rather than other properties of water furnish the effective stimulus, in which event hydrotropism would properly be regarded as a special kind of chemotropism.

Aerotropism.—Under the conditions prevailing in some of the experiments above described, there was doubtless an inadequate supply of oxygen, as when a medium poor in oxygen was enclosed between impervious plates. There was then a very decided tendency for the hyphae to turn toward the edges of the plates. The observation of this phenomenon from time to time suggested that the fungi might show an aerotropic sensibility, either as a positive reaction to oxygen, or as a negative reaction to carbon dioxid.

In order definitely to test the matter, experiments were arranged

in which the growth toward holes in mica plates could be observed when the plates separated normal non-nutrient gelatin from non-nutrient gelatin saturated with carbon dioxid on the one hand, and from normal non-nutrient gelatin on the other. The fungi used were *Penicillium glaucum*, *Sterigmatocystis nigra*, *Mucor mucedo*, *Botrytis vulgaris*, *Monilia fructigena*, *Monilia sitophila*, and *Phycomyces nitens*. In no case was the percentage of turning toward the carbon dioxid gelatin greatly different from that toward the control gelatin.

As a further test, a layer of gelatin containing spores was placed below a perforated mica cover for a Van Tieghem cell, and a perforated mica plate, small enough to fit inside the cell, was placed below the gelatin. A layer of normal gelatin was spread below this last plate, and a layer of carbon dioxid gelatin above the cover. This preparation was sealed to the cell rim, and the whole placed under a bell jar practically filled with carbon dioxid and kept at a room temperature of 21–24° C. Efforts were made to have the moisture conditions equal within and without the cells; and the exposed gelatin layers, which served very well as indicators, showed no difference in this respect until after the observations on the majority of the preparations had been made, although there was drying of the outer gelatin layer by the time the more slowly growing fungi had reached the proper stage. The same fungi were used in this experiment as in the preceding one, with the addition of *Mucor stolonifer*. In most instances the turning toward the gelatin containing carbon dioxid and exposed to an atmosphere of carbon dioxid, was as great as toward normal gelatin; the growth, however, was less vigorous in the former case. In those preparations in which there was less turning toward the carbon dioxid gelatin, this gelatin had become evidently rather dry.

It is to be concluded, therefore, that the observed turning toward the edges of preparations is not due primarily to aerotropic sensibility. The experiments also negative the supposition that the observed repellent influence of growing hyphae may be due to the consumption of oxygen or to the production of carbon dioxid by the fungus, or to both.

Osmotropism.—Notwithstanding the fact that osmotropism is

intimately associated with chemotropism, and that many of the tests for the latter are in equal measure tests for the former, direct tests were made by growing the fungi in media of higher osmotic pressure and of lower osmotic pressure than the test media, as well as in an isosmotic medium; glycerin, a good nutrient substance, and yet a substance reported by MIYOSHI to be neutral in its chemotropic effect, was used to give the desired concentration to the culture media. The series failed to show that the concentration of the culture medium has an effect upon the amount of turning. However, no excessively high concentrations of mineral salts were used.

Other tropic phenomena.—Under conditions that would favor a manifestation of geotropism and of thigmotropism, there was no indication that these are concerned in determining the direction of growth of the fungi. The effects of light and of heat were in no way tested, but they probably do not enter as factors.

Biological significance.—The conclusions reached in these studies may be found to have a somewhat important bearing upon the biological problem of infection by parasitic fungi. In the absence of any experimental investigation, nothing definite can now be said. It would seem, however, that the drying of dew and other surface moisture in which spores had gerimnated, might be a condition favoring the hydrotropic turning of the germ-tubes toward the stomates, especially if the cells within are over-distended with water, which has frequently been observed to be a condition favorable for infection; if the germ-tubes are numerous in the vicinity of a stomate, the repellent influence of these upon one another would cause some to seek the unoccupied region within the stomate. At all events the phenomenon of the entrance of germ-tubes, whether by way of the stoma or through the cuticle, is a complex one, of which many factors remain undetermined. That mere entrance is probably not due to specific peculiarities, either of host or parasite, is evidenced by the recent work of Miss GIBSON which has been mentioned by MARSHALL WARD (**33**). Miss GIBSON found that spores of various members of the Uredineae sent their germ-tubes readily into the stomates of plants widely different from their hosts, and which they were unable to infect. MARSHALL WARD met with numerous instances of the same phenomenon. This would indicate

that the entrance of a hypha into a stoma is merely a preliminary act, distinct from infection proper, and controlled by general conditions, while the fate of the hypha after its entrance is determined by complex reactions between parasite and host, which are largely specific in their nature. In the light, then, of known facts, no simple explanation, such as the theory of chemotropism due to the presence of specific chemical compounds, is adequate. Chemotropism may possibly be one factor in the complex phenomenon, but it is certainly not the predominant factor.

CONCLUSIONS.

Various tests upon a number of fungi failed to indicate the existence of any definite chemotropic sensibility to nutrient substances or other chemical compounds in solution. If positive chemotropism exists, it is less prominent than other tropic phenomena involved, and was obscured by them.

Those substances which furnished nutriment to the fungi caused a decided growth, often with thickening of the hyphae and an increased branching; but they did not cause a more marked turning of the hyphae toward the diffusion centers than did non-nutrient and toxic substances.

All of the fungi tested show a tendency to turn from a region in which hyphae of the same kind are growing toward one destitute of hyphae, or in which the hyphae are less abundant. The turning toward a medium in which mycelium has grown, but from which the mycelium has been removed, is less marked than that toward a medium in which no mycelium has grown. This may be regarded as a negative reaction to stimuli from chemical substances, which owe their origin in some way to the growing fungus.

Various fungi show a positive hydrotropism; but an overabundance of moisture may cause a negative reaction in certain fungi.

The changing of the direction of growth of fungous hyphae is a complex phenomenon in which at least two factors, cytotropism and hydrotropism, are concerned. Since the complete elimination of neither of these factors is possible, all tests must be relative, and to that extent unsatisfactory.

It would seem that the reactions of mycelium to various stimuli

are not necessarily the same as the reactions, under similar conditions, of sporangiophores, gametophores, and other specialized parts.

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THE EMBRYOLOGY AND DEVELOPMENT OF RICCIA LUTESCENS AND RICCIA CRYSTALLINA.[1]

CHARLES E. LEWIS.

(WITH PLATES V–IX)

IN June 1903, while collecting liverworts in the vicinity of Ithaca, N. Y., an abundance of material of *Riccia lutescens* was found growing around the edges of dried-up ponds. In some cases the plants formed beautiful rosettes, but usually they grew in irregular clusters, often being so closely crowded together as to cover the ground for several square centimeters.

The individual plants vary greatly in shape and size. The younger light green plants consist of a narrow, thin, ribbon-shaped thallus which has a longitudinal median groove. In the older plants the fore part of the thallus is thickened, very large air cavities being formed. The thallus is attached to the soil by numerous rhizoids from the older part, the apical end being free. On the under side are numerous colorless lamellae.

As the fruiting plant is unknown there is doubt as to the relationship of this species, authorities differing widely as to its status. LINDBERG (**21**) claimed that it was merely a sterile terrestrial form of *Ricciocarpus natans*. UNDERWOOD (**30**) says of it: "approaches certain terrestrial forms of *Ricciocarpus natans*, and possibly derived from that species, but better kept distinct." STEPHANI (**28**) states that it is probably not a Riccia but a sterile marchantiaceous hepatic.

For the purpose of determining the true relationship of the species, Professor ATKINSON suggested the desirability of following the development of the plant through the summer and autumn, and of securing fruiting specimens if possible. He had found young antheridia in plants collected several years before, but had not traced the development further. It also seemed desirable to study the embryology and cytology of the plant if material could be obtained, because comparatively little has been done on these phases of the life history of Riccia.

[1] Contribution no. 106, from the Department of Botany, Cornell University.

BISCHOFF (2) investigated a number of species and settled beyond a doubt the function of the sexual organs. His work was followed the next year by LINDENBERG'S monograph (20) which added little that was new.

The study of the development of Riccia really begins with HOFMEISTER (15), who gave an account of the development of the thallus, sexual organs, and fruit of *Riccia glauca*.

KNY (19) made a careful study of the apical cells and the method of growth of the thallus. He did not secure plants developing from spores but used delicate thalli which had grown crowded together and did not bear sexual organs in the younger parts, so that the regular order of cells was not disturbed. He discovered the origin and manner of growth of the ventral scales and described the development of the sexual organs. Although HOFMEISTER believed that young antheridia and archegonia could not be distinguished, KNY points out that they are distinct after the first walls are formed.

LEITGEB (22) gives a complete account of the method of growth of the thallus in the Ricciaceae. His study of the sexual organs and fruit was in many cases incomplete on account of insufficient material.

BIOLOGY OF RICCIA LUTESCENS.

The account of the biology of *Riccia lutescens* given here is based on field observations extending through two years, together with experiments and observations upon plants kept growing under favorable conditions in the greenhouse and laboratory.

The first observations were made late in June. At that time the plants were growing upon the mud around the edges of ponds. Some of the thalli were very small and delicate, appearing merely as green specks on the mud, while others, which seemed to be older, had the ribbon-shaped form and thickened apical end already described (*figs. 1–3*).

Material was collected and examined from time to time during the summer and autumn, with the expectation of finding plants bearing the sexual organs, because the statement is usually made that the species of Riccia fruit in summer and autumn when growing on the soil. The plants continued to grow well vegetatively throughout the summer, when they were in such a location that they were

supplied with sufficient moisture. In some cases the mud became so dry and hard that the plants were killed, but whenever they were sheltered by a stone or other object, or were growing on the sides of holes, such as cattle tracks, they grew well.

In October all but the youngest and most crowded plants showed the typical *Riccia lutescens* form. At this time young antheridia were found. Material was now collected and fixed from time to time for the purpose of studying the development of the sexual organs. In very few cases were archegonia found in plants collected in autumn. A few young stages were found in plants collected late in November, at which time the older antheridia were almost mature. No further development took place out of doors until spring, because the plants became covered with snow, or with water by the filling up of the ponds, and remained so until April. A quantity of the plants were kept growing on the soil in the greenhouse through the winter, and developed mature sexual organs long before spring. Plants taken from under water in March, just as the ice was going out of the ponds, showed exactly the same form as in November, and little or no further development had taken place. So it seems that the development depends to some extent on temperature, and might be expected to vary with different conditions of climate. A warm winter, in which some growth might take place, would in all probability hasten the development of the sexual organs. Another point of interest is that the submerged plants did not seem to have been injured.

A quantity of material still attached to the soil was taken from under water late in March, and was kept growing in shallow pans in the laboratory so that it could be kept supplied with a sufficient quantity of water for growth but not enough to flood the plants. This was done in order to determine whether the plants would continue the development of sexual organs and fruit in the same way when supplied with a limited amount of water and growing on the soil, as when supplied with a large amount of water which would tend to cause them to break loose and float. It was found that the plants growing on the soil did produce fruit abundantly and at the same time as those growing under natural conditions. The archegonia begin to develop in April in the same thalli which have

produced antheridia, and all stages are found by May 1. About this time fertilization takes place, and by May 25 all stages of sporophyte are found. The arrangement of the sexual organs in the thallus is shown by *figs. 7–11*.

The vegetative growth is very rapid during April and May, the thallus becoming broad and branched by the increase in the number of growing points. The narrow older part by which the thallus is attached decays, and the younger part bearing the sexual organs and sporophytes is set free and floats upon the water. When the plants are supplied with a large amount of water changes take place in the lamellae. They grow to great size and become purple. In the floating thallus decay of the older part continues; the part bearing the antheridia first disappears, then the part bearing the sporophytes, and finally the growing points may be separated, one thallus thus giving rise to several new individuals. In most cases observed the decay of the older parts in floating plants did not advance so far. The plants were carried up around the edge of the pond by the waves, and as the water went down were left stranded upon the mud. When the thalli settle down upon the mud, the large ventral plates wither, and rhizoids are put forth which in a few days attach the thallus to the soil. Growth now continues at the growing points, so that new branches are produced which form rosettes.

When the thallus is injured at this time new plants are immediately produced from the cells of the apical region. This was first observed in plants injured by being covered with mud, in which case slender delicate outgrowths were produced (*fig. 4*). Other plants injured by snails soon developed long slender plants (*fig. 3b*). Thalli were cut into pieces to determine whether other cells would show the same plasticity, but new plants were produced only from cells near the growing point. VÖCHTING (**33**) found in Lunularia that regeneration takes place from cells in various parts of the thallus, but this does not seem to be true of *Riccia natans* under the conditions in which I have studied it. Large numbers of the plants which were left upon the mud when the water went down were injured by cattle coming down to the ponds to drink. Later in the season the cattle tracks were lined with young, green, ribbon-shaped plants

which were outgrowths from the growing points of the older plants. The cattle tracks serve a good purpose, as the young delicate plants are shaded and protected to some extent during the dry season. Two forms of the thallus are produced by the different methods of propagation. In the one case the thallus after it becomes attached to the soil continues its growth, branches and forms a rosette, while in the other case the thallus is injured, and very delicate forms are produced. When large numbers of the floating thalli are deposited near together and are then injured, we find the irregular clusters of plants which have been described in the first paragraph.

The thallus of this plant during the floating period bears such a striking resemblance to *Ricciocarpus natans* that one is led to the conclusion that *Riccia lutescens* is only a ground form of *Ricciocarpus natans*. Since the beginning of this study and after it was well under way, a paper was published by GARBER (11) which dealt with the life history of *Ricciocarpus natans*. Several points in the biology of the plant as given by GARBER differ from those found to obtain at Ithaca, and since the structure of the thallus as well as the embryology is conclusive proof that the two forms are the same species, it seems proper to call attention to these differences and then to give briefly the embryology before taking up the other phases of the study.

The greatest difference in our observations lies in the relation of the supply of water to sexual reproduction. GARBER states that *Ricciocarpus natans* as it grows at Chicago spends its entire life, from the germination of the spore to the production of spores, in the floating state, and that the occasional fruiting plants found upon the soil in summer are plants in which the sexual organs developed and the sporophytes began their development while the plants were floating. He observed no case in which sexual organs were produced on plants growing upon the soil and states that *Ricciocarpus natans* has not yet acquired the power to reproduce sexually when growing upon the soil. The sexual organs develop in April.

The plants at Ithaca, however, spend the greater part of their life upon the soil and only float upon the water for a few weeks at the fruiting period. The sexual organs begin to develop in autumn while the plants are on the soil and plants kept on the soil and sup-

plied with a limited amount of water developed fruit. At the time when the antheridia begin to develop the gametophyte is under favorable conditions for vegetative growth, but is not supplied with an abundance of water. The soil is moist and the conditions are such as would favor the growth of a terrestrial form like Marchantia.

The plants seem especially adapted to spend the winter submerged and do not perish under such conditions. The fore part of the thallus contains very large air cavities and thus the tissue is aerated. It is well known that certain higher plants which grow in wet situations have large air spaces in the tissue, and GANONG (**12**) calls attention to the fact that those marsh plants which are submerged for a portion of the year are able to survive on account of their capacity for air storage. About May 1 the older part of the thallus, which is narrow and thin, has decayed, and the younger parts, bearing the sexual organs, is set free and floats. GARBER points out that when land forms are placed upon the water only a small portion of the apical end remains above the surface, while the older part of the thallus extends into the water and decays. This is true of plants taken from the soil in summer, but in the spring when the free part of the thallus is thick and contains large air cavities, it floats readily. The length of the floating period depends of course upon the conditions of the pond. In some cases the plants may very soon be carried up around the edge of the pond and deposited on the mud, but floating forms are usually found until the ponds are almost dry. In the case of ponds which do not become dry in summer, both forms would be found. The floating period affords an excellent means for distribution.

When the plants grow upon the soil and are not protected during the winter by a covering of snow or water, they are usually killed by freezing, but in some cases plants which were brown and seemed to be dead produced new thalli from the growing point. The young delicate thalli are well adapted to tide over the dry season, because they can live with a less supply of water than would be needed by the older plants.

RELATIONSHIP OF THE SPECIES.

The form usually described as *Riccia lutescens* should be regarded as a ground form of *Ricciocarpus natans*. Both in the field and in

cultures in the laboratory the forms have been observed showing the transition. There can be no doubt that the plant which I have described is the true *Ricciocarpus natans*, and the description of the ground form as a distinct species came about naturally from the conditions of its growth. In such ponds as have been observed here, the water is high in April and May, so that the floating plants are carried up around the edge and left on the soil. In June or July the water has entirely disappeared from the pond and the only plants found are the slender ribbon-shaped ones which have developed from the floating form. In my first summer's collecting, when the ponds were dry by the last of June, I saw not a vestige of the old *Ricciocarpus natans*, and felt sure that the plants collected were *Riccia lutescens*. It seems possible that the plant was first described as a distinct species from material collected under similar conditions, because it is said to occur in dried up ponds and ditches. If in summer and autumn some water were present, so that some of the typical *Ricciocarpus natans* would be found floating, the origin of the ground form might readily be seen, but in such a case there might be failure to associate the ground form with *Riccia lutescens*. Only by following the development and observing the transition of one form into the other under different conditions of growth can the true relationship be determined. My observations have convinced me that *Riccia lutescens* is only a ground form of *Ricciocarpus natans* and should not be regarded as a distinct species.[2]

The plant now known as *Ricciocarpus natans* was formerly regarded as a Riccia. In the structure of the thallus Ricciocarpus is more complicated than the species of Riccia. The most important taxonomic characters, however, have been the arrangement of the sexual organs and structure of the sporophyte.

HOOKER first found fruiting plants in dry specimens sent to him by TORREY from New York in 1824. BISCHOFF found fruiting plants in the autumn of 1829 near Heidelberg, and describes antheridial plants, but his figure of the antheridium is not very convincing,

[2] Having determined the ground form as *Riccia lutescens*, specimens were sent to Professor A. W. EVANS in October 1904. He considered that we were right in referring the plants to that species, but stated the views of different authorities in regard to the status of the species.

as it looks more like the mass of tissue which projects up as a ridge into the median groove, the cells being quite too large for those of an antheridium.

Although HOOKER considered that the plant should remain in the genus Riccia, CORDA placed it in a new genus, Ricciocarpus, on the basis of HOOKER'S description and figures which were taken from dried material. CORDA'S figures are copies of HOOKER'S. BISCHOFF held that there was no real basis for the change, as the mature sporophyte does not differ from that of other Riccias, the separation being based on the mistaken notion that the capsule walls disappear entirely at maturity, and that the genus Riccia should not be divided on account of differences in the thallus brought about by the different conditions under which the plant grows, since the method of fruiting is the same in all the species.

LEITGEB regarded Ricciocarpus as a distinct genus, on account of the more complex structure of the thallus and the grouping of the sexual organs. He thought that the antheridia were collected into groups similar to those in the Marchantiaceae, but GARBER'S results and my own show that LEITGEB was not correct, and that the antheridia actually form only one group. The archegonia are also arranged in a definite part of the plant in one group.

The question now arises whether this is a more advanced condition of development than is found in species of Riccia. In the lower species of Riccia, the sexual organs are said to be indiscriminately scattered over the surface of the thallus, while in *Riccia fluitans* a regular alternation of single antheridia and archegonia is described. CAMPBELL, in discussing the arrangement of sex organs in Riccia, says that in the two forms which he studied, *Riccia hirta* and *Riccia glauca*, he found as a rule that several of one sort or the other would be formed in succession. I have observed the same in *Riccia crystallina*, although the older sporophytes appear scattered in the thallus. LINDENBERG described the fruit of *Riccia crystallina* as scattered, but the antheridia are described and figured as being in a group along the middle part of the thallus. He described and figured the fruit in *Riccia glauca* as being sometimes in rows and sometimes scattered. Most of the figures show them in more or less perfect rows along the longitudinal axis.

In *Riccia minima*, LINDENBERG (**20**, p. 429) describes and in *pl. 20* figures the antheridia as arranged in two rows, one on each side of a median groove. In *Riccia bulbosa* the antheridia are along the median groove for its entire length, sometimes in pairs and sometimes far apart. *Riccia Bischoffii* has the antheridia in two or three rows in the thallus.

It seems highly probable that a careful study of a large number of species of Riccia by modern methods would show that in many of them there are produced groups of antheridia and archegonia in distinct parts of the thallus.

Since the characters upon which the genus Ricciocarpus has been based, with the single exception of the structure of the thallus, have been found wanting, it seems to me that there is not sufficient reason for retaining the genus. The thallus varies in form according to the supply of water, and when growing on the soil has been called a species of Riccia. Many plants assume quite different forms when growing under different conditions, but the different forms are not regarded as species.

We should then write:

RICCIA NATANS L. Syst. Veget. 956. 1774.—Bischoff, Nova Acta Acad. Caes. Leop. Carol. **17**: 2. 1835.—Lindenberg, Nova Acta Acad. Caes. Leop. Carol. **18**:—1836.—Sullivant, Gray's Manual 2ed. 1856.

Ricciocarpus natans Corda, Opiz Naturalischentausch. 1829.—Leitgeb, Die Riccien, Unters. Lebermoose 4: 1879.—Lindberg, Revue Bryol. **9**:82. 1882. (Includes *Riccia natans* L. and *Riccia lutescens* Schw.)—Schiffner, Engler and Prantl. 1893.—Campbell, Mosses and Ferns. 1895.—Underwood, Systematic Botany of North America. Hepaticae. 1895.—Garber, BOT. GAZETTE **37**:101–177. *pls. 9–10.* 1904.

Riccia lutescens Schw. Specimen Fl. Amer. Sept. Crypt. 26. 1821.—Lindenberg, Nova Acta Acad. Caes. Leop. Carol. **18**: *pl. 26.* 1836.—Sullivant, Mem. Amer. Acad. II. **4**: *pl. 4.* 1849.—Sullivant, 2d ed. Gray's Manual 684. 1856.—Underwood, Systematic Botany of North America, Hepaticae. 1895.

Riccia velutina Hooker (in part) Ic. Pl. *pl. 149*: founded on sterlile thalli of *Riccia lutescens* and fertile thalli of *Riccia crystallina*, according to Sullivant, Gray's Manual, 1856.

EMBRYOLOGY.

Material for study was collected during the autumn and spring, and fixed very satisfactorily in 1 per cent. chromacetic acid or in chromosmacetic.

The large air cavities prevent the penetration of the fixing fluid, to overcome which the pieces were submerged by means of cotton plugs. After dehydration the material was passed through chloroform into paraffin. Sections were stained with the triple stain of Flemming or with Heidenhain's iron-alum haematoxylin.

SEXUAL ORGANS.

Young antheridia were found in October. They begin to develop while the plants are young and growing on soil not supplied with a large quantity of water, although the conditions for vegetative growth are good. At this time the thallus is ribbon-shaped, with a thickened apical end and a longitudinal median groove, the thallus in cross-section having about the shape of an inverted Y with a ridge of tissue between the arms (*fig. 9*). Very few plants are found which do not produce antheridia. The archegonia develop later in the same thallus. At first there seemed to be in this a distinction between *Riccia lutescens* and *Ricciocarpus natans*, because *Ricciocarpus natans* has been described by SCHIFFNER, LEITGEB, and CAMPBELL as being strictly dioecious, but the work of GARBER shows conclusively that it is monoecious. The earlier observers state that Ricciocarpus fruits in autumn, so it seems probable that their material was collected after the older portion of the plant had decayed, leaving only the portion bearing sporophytes.

The antheridia are produced in acropetal succession in three to five rows (*figs. 10, 11*).

The antheridium develops as has been described for other species of Riccia. A superficial cell on the floor of the dorsal furrow just back of an apical cell protrudes above the surface and is cut off by a horizontal wall. The outer cell increases in size, and is divided by three or four cross walls, then a longitudinal wall is formed dividing the young antheridium into two equal parts: this is followed by a second longitudinal wall perpendicular to the first. Then periclinal walls are formed which cut off the single layer of cells which form the wall of the antheridium. The cells in the center now undergo repeated divisions until a very large number of cells is formed. Each of these cells is almost cubical in form and in Riccia has been described as producing a single spermatozoid,

KNY (19). The mature antheridium is a short stalked oval body with a conical apex.

As the antheridium develops, the vegetative tissue grows up and surrounds it so that it is enclosed in a cavity which opens into the dorsal furrow. This cavity is formed in the same way as the air spaces of the thallus. The apex of the antheridium is a little below the floor of the dorsal furrow and the sperms escape through the neck formed by the surrounding tissue. Although the antheridia begin to develop in autumn, they are not mature until the following spring, because the growth is checked by the cold. Plants kept in a warm place produced mature antheridia during the winter.

A series of archegonia is developed which is a continuation of the series of antheridia (*fig.* 7). The archegonium is at first superficial on the floor of the dorsal furrow. Later it becomes enclosed in a cavity by the upward growth of the vegetative tissue as in the case of the antheridium except that the neck of the mature archegonium protrudes above the bottom of the furrow. The origin of the archegonia side by side at the bottom of the dorsal groove is shown in figure 9. In this way three to five rows are formed and later a large number of sporophytes are found in each thallus.

The archegonium develops in general as has been described by JANCZEWSKI (17). My observations confirm the account given by GARBER for *Ricciocarpus natans*, as a comparison of the figures will show, so it is unnecessary to describe the development here.

About the time when the archegonia are mature, cross-sections of the thallus show numerous, delicate, almost hyaline, club-shaped hairs extending up from the floor of the median groove. Each hair consists of a stalk of two or three short, narrow cells with a much larger cell at the free end. These hairs bear a striking resemblance to paraphyses(*fig.* 78). LEITGEB (22, p.31) describes "papillae" which grow up from the bottom of the groove and regards it as highly probable that it was the dried remnants of these which LINDENBERG observed when he wrote: "Sporangium vor aussen mit kleinen unregelmässigen braunen Schuppen bedeckt ist, die Fragmente einer zersprengten früheren Hülle zu sein scheinen." As the hairs become older they become brown and break down so that they

would give much the appearance described by LINDENBERG. We know now that the sporophyte has no *Hülle* or sheath.

SPOROPHYTE.

The development of the sporophyte agrees with the account given by CAMPBELL (3) for Riccia and by GARBER for *Ricciocarpus natans*.

The first division is usually transverse but may be oblique (*fig. 21*). The next wall may be perpendicular to the first so as to form a quadrant (*fig. 22*), or parallel to it, producing a row of cells. Divisions take place in all directions after this until an almost spherical mass containing several cells is formed. Then the amphithecium becomes distinct as a single layer of cells enclosing the spore producing cells. The growth takes place rapidly but the divisions of the cells are not simultaneous, usually only a few dividing cells being found in a sporophyte.

The sporophyte continues its growth until a solid mass of three to four hundred cells is produced. Then the calyptra and amphithecium expand and the spore mother-cells becoming free separate from one another and become rounded. From the surrounding cells, which are richly stored with food, there is secreted a large amount of nutritive material which fills the space around the mother-cells, giving them favorable conditions for growth (*figs. 25, 26*). The spore mother-cells increase rapidly in size and again fill the cavity. That part of the nutritive material not absorbed by the spore mother-cells is pressed into thin plates between them. This material takes a deep blue stain with gentian violet. A fuller discussion of the spore mother-cells and of their division to produce the spores will be given in another place.

Before the spores are mature the inner layer of the calyptra collapses. The amphithecium is distinguishable until the spores are almost mature. The outer layer persists but the cells are usually shrunken. The contents of these cells is no doubt used up to supply the growing spores with nourishment. All of the spore mother-cells produce spores, there being no sterile tissue in the form of elaters. In discussing the simple form of sporophyte of Riccia, GARBER considers that the absence of sterile tissue is to be associated with the habit of the plants; since there is not much chance for the attachment of an independent sporophyte, there is no sterile tissue in

the form of a foot. When we consider the fact that some other Hepaticae which have the foot well developed grow on very wet soil and require as much moisture for their development as do some of the species of Riccia, this theory does not seem entirely convincing.

The sporophyte develops during May and June. A given sporophyte requires about three weeks for its development.

SPOROGENESIS.

Usually the most favorable cells for the study of cytological details are the spore mother-cells. Their large size, abundant contents and active growth at the time when divisions are taking place, permit good results in fixation. *Riccia crystallina* has furnished the most satisfactory material.

In July, 1903, an abundance of fruiting *Riccia crystallina* was found growing on the mud on the bottom of a dried up pond not far from the ponds where the form known as *Riccia lutescens* was growing. This species had never been collected in this region before. Having so determined the plant, I referred specimens to Prof. A. W. EVANS who confirmed my determination. He says: "Apparently this species represents an addition to the hepatic flora of New York. I find no mention of it in local lists of New York plants and there are no specimens of it from your state in my herbarium."

These plants had been growing under favorable conditions, as the pond had not contained much water at any time during the spring. The thalli formed rosettes growing so close together as almost to cover the ground. The number of fruiting plants was very striking, as it seemed impossible to find a single sterile plant. All stages in the development of the sporogonium and spores were easily obtained, and some stages in the development of the sexual organs, but changes were taking place very rapidly and the younger stages were of comparatively rare occurrence. The development of the sexual organs and fruit agrees with that of other species of Riccia. Each thallus produces several sporophytes which are easily recognized when mature as small black spherical bodies imbedded in the tissue.

These plants continued to develop and produce sporophytes for only a short time after they were discovered. The month of July

was the most favorable time for the collection of material showing karyokinesis in the spore mother-cells. During August, the spores became mature and the thalli broke down. No good specimens could be collected after August 25. This differs from what has been observed for some other species of Riccia, which are described as withstanding long periods of drought, the thalli continuing their growth again when supplied with moisture. (CAMPBELL, **3**.)

During the following winter this pond became filled with water and did not become dry until late in the summer, so that only a few plants were found as compared with the large number of the preceding year. This made a difference in the time of fruiting. In September the sporophytes were in about the same stage of development as in July of the preceding year. This may explain why different authors give different seasons for the fruiting of Riccia. It seems that conditions of temperature and water supply exert such an influence that in the same species and locality the time may vary considerably from year to year. In general, I think it may be said that good conditions for vegetative growth will hasten rather than retard the fruiting of Riccia.

The thallus of *Riccia crystallina* is small and thin; its surface presents a series of wide depressions separated by thin lamellae; and there are no ventral scales. The fixing fluid easily penetrates and the spore mother-cells are usually well fixed.

The development of the spore mother-cells agrees with the account given for *Riccia natans*, but there is not such a large number produced in a sporogonium. When the spore mother-cells come to lie loosely in the sporogonium, they are surrounded by nutritive material. The mature spore mother-cells are then generally spherical, but they may be elliptical or so angular by crowding as to look like a tissue. The contents of the spore mother-cells of Riccia has been described as granular by CAMPBELL (**3**) but the structure of the cytoplasm in *Riccia crystallina* is a fine reticulum with the granules occurring usually at points of intersection of the fine threads of the network. The older spore mother-cells as well as the mature spores contain considerable oil.

In the nucleus of the spore mother-cell the chromatin is scanty and is irregularly scattered on a fine linin network. No nucleolus

has ever been observed (*fig. 34*). When the nucleus is preparing for division, the chromatin leaves the linin network and collects into several bodies which soon move together to form one irregular mass. I regard this as the synapsis stage. Such bodies of chromatin have been found often and in cells which seemed to be well fixed so it seems to represent a stage in the preparation for division and not to be a result of shrinkage as has been suggested by certain authors for other plant cells in which the same condition has been observed. The body of chromatin occupies a position at one side of the nucleus, and the rather large nuclear cavity appears hyaline. There can be little doubt that the body described by CAMPBELL (**3**) as a nucleolus is really the entire mass of chromatin in the synapsis stage.

From this mass of chromatin a short thread develops which later segments to produce the chromosomes (*fig. 35*). The small amount of chromatin present here makes the details very difficult to determine. The four chromosomes, easily counted here as well as in the nuclear plate and on the way to the poles, are very small and appear almost spherical when on the spindle although they are short, thick, curved rods.

The development of the spindle is not easily observed. Divisions take place almost simultaneously in all the cells of a sporogonium and the changes are very rapid. By far the commonest stage of division is that in which the chromosomes are in the nuclear plate (*fig. 38*). Neither centrosomes nor centrospheres occur in the spore mother-cells of either *Riccia crystallina* or *Riccia natans*. Around the nucleus preceding the formation of the spindle, there is an accumulation of material, apparently composed of fine fibres. The nucleus elongates, becoming somewhat elliptical but not sharp pointed. The fibres about the nucleus do not give the appearance of centrospheres but are like the weft of kinoplasmic fibres described for certain pollen mother-cells (*figs. 36, 37*). It has been impossible to find any nucleus which showed anything resembling a multipolar spindle. The poles of the spindle are probably determined by the elongation of the nucleus at an early stage in the spindle formation. The spindle is composed of very fine fibres, some of which extend from pole to pole, while others extend from the poles into the cytoplasm, reaching almost to the nuclear plate (*fig. 38*). The

mature spindle has very broad poles and its formation does not seem to have been controlled by a centrosome or a centrosphere, as a comparison of the spindles of the spore mother-cells with those of the cells of the antheridium makes clear.

The minute chromosomes separate, four going to each pole, after which a cell plate is formed in the usual way (*figs. 40, 41*). The daughter nuclei do not come to a true resting stage. The chromatin is scattered in almost spherical bodies in the hyaline cavity of the nucleus, which do not represent the individual chromosomes, as their number and size vary considerably (*fig. 42*).

The second division takes place in much the same manner as the first. The spindles are arranged with their long axes parallel to the first cell plate, so that the cell plates formed in these spindles are almost perpendicular to that formed in the first division (*figs. 43–47*). The latter does not disappear during the second division but remains and the walls separating the spores are laid down here (*fig. 47*). The walls separating the cells of the young tetrad are thin and delicate, but in the mature spore the outer layer of the wall becomes thickened and folded. The mature spore is almost black, and its contents are largely oil. When carried through chloroform into paraffin and sectioned, the spores seem to have only scanty granular contents, due to the fact that the oil has been removed in the process. The nucleus is very small.

During the winter and spring following their development, unsuccessful attempts were made to germinate the spores. It may be that they had been allowed to remain dry too long before they were moistened, for in nature they would not be dry very long even in tiding over a dry season.

The spore mother-cells of *Riccia natans* do not furnish such satisfactory material for study as do those of *Riccia crystallina*, because the large air cavities of the thallus prevent the penetration of the fixing fluid and so the spore mother-cells often shrunk. A sufficient number of good preparations was secured, however, to show that the process of division does not differ from that of *Riccia crystallina*.

SPERMATOGENESIS.

The development of the spermatozoids has been treated by a number of investigators, among them, CAMPBELL (4), LECLERC DU SABLON (23), GUIGNARD (14), SCHOTTLANDER (27), and STRASBURGER (29). It will be observed that most of these papers were published before methods of preparing material for study were so well developed as at present. The work of BELAJEFF (1) confirmed by that of STRASBURGER (29) shows that the spermatozoid in the Hepaticae consists not only of the metamorphosed nucleus but also of the cytoplasm.

IKENO (16) not only confirms the view that the spermatozoid consists of cytoplasm as well as nucleus but also discusses the development of the cilia and the homology of the blepharoplast and centrosome of *Marchantia polymorpha*.

He finds that the body which becomes a blepharoplast in the developing spermatozoid appears in the earlier nuclear divisions of the antheridium and functions as a centrosome. It is, however, not permanent, but appears at the time of nuclear division and disappears during the process, so that it is not found in the daughter cells until about the time for the formation of the spindles of their division. After the last division which gives rise to the cells that develop into the spermatozoids, the body does not disappear but remains and becomes a blepharoplast. IKENO argues from this that the centrosome and blepharoplast are homologous. He has good grounds for such an argument in the case of *Marchantia polymorpha*, because centrosomes have been reported also in the vegetative cells of that plant, by MOTTIER (24) and by VAN HOOK (32). In other plants which have the blepharoplast, centrosomes are not found, and the body appears in only one or two divisions before the formation of the cells which produce the spermatozoids.

MOTTIER (26) in discussing IKENO'S paper raises the question whether the bodies which IKENO has figured as centrosomes are in some cases more than ordinary granules such as appear in the cytoplasm of other cells in which centrosomes are known to be absent. IKENO has pointed out, however, that the cytoplasm of these cells is very finely granular, there being no other bodies in the cell which bear any resemblance to the ones figured as centrosomes. He also calls

attention to the fact that centrospheres have been described in dividing spore mother-cells of *Pellia epiphylla*, by FARMER (**6, 7, 8, 10**) and by DAVIS (**5**). The occurrence of centrospheres here has been questioned, however, by GRÉGOIRE (**13**). In a recent paper, FARMER (**9**) reports centrospheres and occasional centrosomes in the spore mother-cells of *Aneura pinguis*.

In order to get good results in *Riccia natans* it is necessary to fix the material when growing rapidly. About equally good results were secured with chromacetic acid and with Flemming's weaker solution. The sections were stained with anilin safranin and gentian violet. It was found best to stain deeply in gentian violet and then to wash out carefully. In this way all details can be brought out clearly, although IKENO did not find it good for Marchantia.

The development of the antheridium has been described. When almost mature it consists of a large central mass of cubical cells surrounded by a wall one cell in thickness (*fig. 33*). In preparations from plants in which some antheridia are mature, one finds several stages in the development. The nuclear divisions do not take place simultaneously throughout an antheridium but usually all the cells of one of the segments marked out by the first walls dividing the antheridium, show the nuclei in the same stage of karyokinesis. In the most favorable preparations, therefore, one may find several stages of division in the same antheridium.

The cells of the young antheridium are almost cubical, with finely granular cytoplasm. The nucleus is rarely exactly spherical and has a rather thick membrane. The chromatin is in an irregular central mass, made up of a number of pieces. A nucleolus cannot be distinguished. The cavity surrounding the chromatin is large and hyaline (*figs. 53, 54*). In some cases a large number of small bodies of chromatin were found scattered irregularly in the nuclear cavity. The number of chromosomes is four. It seems that the nuclei in the young rapidly growing antheridium rarely come to a typical resting stage.

The question of the presence or absence of centrosomes in the cells of the young antheridium was taken up carefully, because previous observations on the karyokinetic figures in the sporophyte cells and spore mother-cells have convinced me that no such body

appears there. On the other hand centrosome-like bodies appear in the cells of the older antheridia at the time of nuclear division. There can be no doubt that these are distinct bodies, and they cannot possibly be interpreted as accidental granules in that position. In some of my preparations hundreds of cells showing them are found on a single slide, and they are so distinct that the preparation could easily be used for class demonstration. These bodies appear in the cells of the antheridium in early stages of its development. I have been unable to determine whether they appear in the earliest cell divisions but they appear in the antheridia which consist of only a few cells. They are not permanent, but disappear and arise anew with each division.

IKENO regarded it as highly probable (though unable to state this positively) that in Marchantia these bodies were of nuclear origin. He figures a small spherical body inside the nuclear membrane, which in a later stage is found outside the membrane. This body then divides into two, which arrange themselves on opposite sides of the nucleus. If the bodies have their origin as one, which later divides as described, they act as do the centrosomes which have been described for other plants.

In *Riccia natans*, nothing has been observed to indicate that the body is of nuclear origin, except that it stains in much the same way as the mass of chromatin in the nucleus. In some of my preparations a single body has been observed near the nuclear membrane (*fig. 53*). These bodies have never seemed so distinct as the ones which appear at the opposite ends of the nucleus and in the poles of the spindle. There is a dark central part, surrounded by a mass of cytoplasm which is more or less irregular but does not give the appearance of distinct radiations such as are described in the centrospheres of certain plants.

When these single bodies were discovered, a careful search was made of the same preparations and of others in which the two bodies were on the opposite sides of the nucleus, in order to discover if possible the intermediate stages which it would seem should appear in such preparations. In cases in which two bodies have been observed, they have always been on opposite sides of the nucleus, or so nearly opposite that the spindle developing between them

might take the curved form shown in *fig. 60*. The origin of the two bodies is of importance in determining the homology of the centrosome and blepharoplast and will be discussed later.

Starting with the stage in which the centrosome-like bodies are on opposite sides of the nucleus, the nuclear division takes place in the following manner. At first the bodies are at a little distance from the nuclear membrane, then the nucleus elongates so that the membrane closely approaches the bodies, becoming somewhat pointed. At the same time one observes that there is a collection of kinoplasm at the poles of the nucleus and extending along the nuclear membrane for some distance. At this time the bodies at the poles do not show radiations in any direction, but are very distinct (*fig. 54*). The spindle is formed from the kinoplasm which has been described, and when formed consists of a few thick fibres which converge at the poles, so that the centrosome-like bodies occupy the position of true centrosomes. About the time when the spindle develops, the chromosomes are formed from the central mass of the nucleus and become arranged in the nuclear plate. They are closely crowded together in this stage, and not so easily counted as when they have moved to the poles. The photograph (*figs. 75, 76*) shows the dense mass formed by the chromosomes when arranged in the nuclear plate. It was impossible to determine how the division takes place in the chromosomes as they move to the poles. The changes take place so rapidly that stages are rarely found in which the chromosomes are on their way to the poles.

The centrosome-like bodies disappear during the division, but it is difficult to say at just what point. *Fig. 56*, a cell taken from an antheridium in which only one or two more divisions will take place, shows the centrosome-like bodies quite distinctly when the chromosomes are almost at the poles, but by the time the chromosomes are at the poles and before the daughter nuclei are formed, the bodies disappear (*fig. 57*).

These bodies are best seen in preparations which have been over-stained and washed out. In some cases my preparations were stained deeply enough to show the spindle and chromosomes well, but only an occasional spindle showed the bodies at the poles. When these slides were over-stained and carefully washed out, the bodies were brought out very distinctly in all cases.

After a large number of divisions has taken place the antheridium consists of nearly cubical cells, each of which has been considered by earlier investigators to produce a single spermatozoid. STRASBURGER (**30**, p. 482) says of *Marchantia polymorpha*: "Die Spezialmutterzellen der Spermatozoiden sind durch fortgesetzte, sich rechtwinklig schneidende Teilungsschnitte angelegt worden." CAMPBELL (**4**) describes and figures the spermatozoid mother-cell of Pellia as producing two spermatozoids. IKENO (**16**) discovered that in Marchantia each of the cubical cells undergoes another division in which the spindles are arranged diagonally, in the earlier divisions the long axis of the spindle being parallel to the long axis of the cell. In this last diagonal division no cell wall is formed between the daughter cells, each of which develops into a spermatozoid. Thus each of the cubical cells produces two spermatozoids instead of one. IKENO cites several cases in which two spermatozoids are produced from a single mother-cell and thinks that this is probably general in the liverworts and mosses.

JOHNSON (**18**) has described a diagonal division of the cubical cells of Monoclea, but he figures a wall separating the two parts of the cell and regards each three-cornered cell as the mother-cell of a spermatozoid. He does not give the details of nuclear division in the earlier stages of the antheridium nor in the formation of the spermatozoid mother-cells.

In the last division of the cells in the antheridium of *Riccia natans* the spindles are arranged diagonally as in Marchantia. This arrangement of the spindles is quite striking. They are larger than in the earlier divisions and the bodies at the poles are very distinct. In some cases the spindles are curved (*figs. 58–60*).

No wall is formed between the daughter cells, each of which develops into a spermatozoid. The centrosome-like bodies do not disappear after this division (*fig. 61*). They remain in the cells, at first near the nuclei. The daughter cells are contracted, occupying the central part of the cell cavity (*figs. 62, 63*). Soon the centrosome-like body moves away from the nucleus toward the end of the cell. Those in the two spermatids may be at the same end or at opposite ends (*figs. 63–67*). When the spermatid has become somewhat rounded, the centrosome-like body has taken its position in contact

with the cell membrane (*fig. 68*). When the cilia appear they are inserted in this very small body so that it comes to function as a blepharoplast. Its small size as compared with that of the cilia of the mature sperm makes it seem probable that some of the material for the growth of the cilia must be drawn from another source than the blepharoplast itself, although it disappears to such an extent that in the mature sperm it cannot be recognized as the point of insertion of the cilia.

The developing spermatozoids of *Riccia natans* do not remain enclosed in the mother-cells until they are mature, but at about the stage represented by *figs. 70, 71* the walls break down and the young spermatozoids lie free in the cavity of the antheridium. Here they seem to undergo considerable growth. The material for this growth is probably derived from the surrounding cells as they become collapsed in old antheridia.

The nucleus of the developing spermatozoid takes a position at one side of the cell and becomes homogeneous. It seems probable that other material than the chromatin of the spermatid nucleus must enter into this part of the spermatozoid, because it is very evident that the body contains more material than would be obtained from the chromatin alone. Soon the nucleus elongates, following the outline of the cell and becoming crescent-shaped (*figs. 71–73*). In some cases, a distinct vacuole occurs in the cytoplasm although this is not always the case (*figs. 71, 72*). The mature spermatozoid becomes long and slender and consists of the nucleus, the material of which seems to have increased in amount, a small amount of cytoplasm, and the cilia which are derived from the blepharoplast and in all probability from a part of the cytoplasm surrounding it. IKENO describes a spherical body which appears in the spermatids of Marchantia before the cilia begin to develop and disappears about the time that changes take place in the nucleus. It has been impossible to find such a body in Riccia, although it would seem, judging from IKENO'S figures, that it could easily be seen if present.

The question of the homology of the blepharoplast and centrosome is one which it seems to me has not yet been settled. In Marchantia, where centrosomes have been reported in the vegetative cells as well as in the antheridium, the evidence seems good that the

centrosome and the blepharoplast are homologous, and this is the conclusion of IKENO. In all other plants in which blepharoplasts are known to occur, centrosome-like bodies are not present in any cell divisions except those immediately preceding the formation of the sperms. That centrosomes occur in liverworts in cells outside the antheridium is open to question. The conflicting reports of those who have investigated *Pellia epiphylla* make it clear that no distinct body occurs there which can be regarded as a centrosome, although aggregations of kinoplasm, called centrospheres by most authors, do occur.

In *Riccia natans*, it seems very evident that centrosomes do not occur in the divisions of the spore mother-cells. The spindle poles are broad, and there is not even a suggestion of a centrosphere such as has been described for Pellia. In the cells of the sporophyte GARBER reports centrospheres but no centrosomes. I have never been able to observe them in my preparations. When the spindle is fully formed there are no fibres radiating into the surrounding cytoplasm (*figs. 48–52*).

Although the thallus of *Riccia natans* does not present favorable material for cytological study, a number of cells showing nuclear division in the gametophyte have been observed near the growing point. The greatest difficulty here is the presence of numerous deeply staining granules in the cell. In some cases granules resembling centrosomes appear at the poles of the spindle, but they do not differ in appearance from the other granules of the cell, and it seems probable that their occurrence here is accidental.

Summing up, we find that in *Riccia natans* centrosomes are not found in the cells of the gametophyte, sporophyte, or spore mother-cells, but that bodies occur in the dividing cells of the antheridium which seem to function as centrosomes. In Riccia and Marchantia, the blepharoplasts certainly have much more the appearance of centrosomes than in any other plants in which blepharoplasts have been described. The bodies have every appearance of centrosomes when at the poles of the elongated nucleus or at the poles of the spindle. Perhaps the strongest objection to regarding these bodies as centrosomes lies in the fact that in *Riccia natans* they occur only in the cells of the antheridium, while the blepharoplasts reported

in other plants appear only in the last two generations of cells concerned in the formation of spermatozoids.

Those who argue in favor of the homology of the centrosome and blepharoplast certainly find their best evidence so far in the liverworts, but it seems to me that this evidence is not conclusive when the bodies occur only in cells of the antheridium.

In those plants in which centrosomes are known to occur, a single body divides to produce two, which arrange themselves on opposite sides of the nucleus (MOTTIER, **25**). IKENO has reported a similar condition in Marchantia. In *Riccia natans*, however, the evidence seems to favor the view that the two bodies arise anew with each division, appearing on opposite sides of the nucleus at the same time. In this respect they behave more like blepharoplasts.

MOTTIER (**26**) in discussing this question has called attention to the fact that it is questionable whether we can speak of organs as homologous which, as such, are without genetic continuity. The question as to whether true centrosomes have genetic continuity has not yet been decided, but it is probable that they do not in all cases.

SUMMARY.

1. *Riccia lutescens* and *Ricciocarpus natans* are forms of the same plant, the former occurring on the ground in summer and autumn when the ponds are dry, and the latter as a floating form. Either form can be changed into the other by altering the supply of water. Therefore, *Riccia lutescens* should not be regarded as a distinct species.

2. The genus Ricciocarpus has been based largely on characters which do not exist. In my opinion, the only real basis for separating it from Riccia is the more complex structure of the thallus. BISCHOFF did not regard this as a good character for the separation of the genus.

3. The plant is monoecious, antheridia and archegonia being produced in definite groups in the same thallus. The sexual organs appear in autumn when the thalli are growing on the ground and complete their development the following April. Abundance of water is not essential to sexual reproduction, as the plants fruit when kept growing on the soil and supplied with a limited amount of

water; therefore the ground form is not sterile, as was the opinion of LINDBERG and GARBER.

4. Plants which have been growing attached to the soil and have been submerged by the filling up of the ponds do not necessarily perish, but are adapted to spend the winter under water and then to break loose by the decay of the older part of the thallus and float upon the water in the spring.

5. The plants are propagated vegetatively by the separation of branches of the thallus, by the decay of the older part, and also by the growth of new plants from cells in the apical region.

6. The sexual organs and fruit of the two species studied agree in their development with the accounts given for the other species of Riccia. There is no rudimentary integument surrounding the archegonium or sporophyte of *Riccia natans*. The sporogonium of *Riccia natans* is larger than that of *Riccia crystallina* and produces a larger number of spores. The only sterile tissue in either is the amphithecium, a single layer of tabular cells surrounding the mass of spore mother-cells.

7. Centrosomes are not present in cells outside the antheridium nor would I interpret any structure observed in the cells of the sporophyte or the spore mother-cells as a centrosphere.

8. Bodies which resemble centrosomes, and which are considered to be true centrosomes by certain authors, occur in the cells of the antheridium. These bodies do not have genetic continuity, but arise *de novo* with each division. They do not disappear after the last division of antheridial cells but remain in the spermatids and later become blepharoplasts.

9. In the earlier divisions of cells in the antheridium, the spindle is arranged parallel to the long axis of the cell, but in the last division, the spindle is placed diagonally in the cell. No wall is formed between the two cells produced by this division, each of which becomes a spermatozoid. Thus two sperms are produced from each cuboidal cell.

10. In the developing sperm, the blepharoplast takes a position on the membrane of the cell and the two cilia grow from it, the nucleus becomes almost homogeneous in structure and crescent-shaped, almost enclosing the cytoplasm. The mature sperm consists of the

nucleus, the cytoplasm, and cilia which have received material for their growth from the blepharoplast and probably also from the material surrounding it.

11. The amount of chromatin in the nucleus is small. There is no nucleolus present unless the masses of chromatin which are found in nuclei which are undergoing repeated division be interpreted as nucleoli.

12. The number of chromosomes is four for the gametophyte and eight for the sporophyte.

13. The cytoplasm of the spore mother-cells appears to be a fine reticulum, in which are numerous granules usually located at the point of intersection of the fibres of the reticulum.

14. The mature spore contains a large quantity of oil together with a small amount of granular matter. The nucleus of the spore is very small.

In conclusion I wish to thank Professor GEO. F. ATKINSON and Dr. E. J. DURAND for valuable advice and assistance during the progress of this study.

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EXPLANATION OF PLATES V–IX.

All drawings, except *fig. 7*, were made with camera lucida. *Figs. 8–52*, with Bausch & Lomb oculars and objectives, as follows: *Figs. 8, 10, 11*, 1 in. ocular, $\frac{2}{3}$ objective; *9* and *25* 2 in. oc., $\frac{1}{6}$ obj.; *19, 21–24, 26, 32, 33*, 2 in. oc., $\frac{1}{12}$ obj.; *12–18, 20, 27–31, 34–52*, 1 in. oc., $\frac{1}{12}$ obj.; *Figs. 53–73* with Zeiss oc. 18, 2^{mm} apochromatic objective, 1.40 aperture.

The figures of plate VI were reduced slightly more than one-half in reproduction.

All figures are of *Riccia natans* except the spore mother-cells (*figs. 34–47*) which are of *Riccia crystallina*.

PLATE V.

FIG. 1. Rosette of plants growing on the soil; *a*, natural size; *b*, enlarged.

FIG. 2. Land plants growing in regular clusters.

FIG. 3. Two plants growing on soil, one of which has been injured and has grown out in an irregular way from the growing point.

FIG. 4. New plants growing from apical cells of old thalli.

FIG. 5. *a*, Decay of older part of thallus of the land form to give the floating form; *b*, plants collected in May. If these thalli should become stranded on the mud and growth should continue rosettes would be formed.

FIG. 6. Plants decolorized in alcohol. The sporophytes appear as chains of dark bodies in the thallus.

PLATE VI.

FIG. 7. Longitudinal section of thallus parallel to the dorsal furrow, showing arrangement of sexual organs.

FIG. 8. Cross-section of thallus showing archegonia.

FIG. 9. Cross-section of thallus, showing the origin of archegonia in rows on floor of dorsal groove.

FIG. 10. Cross-section of thallus, antheridia.

FIG. 11. Longitudinal section of thallus parallel to surface, showing the arrangement of antheridia. Archegonia have not begun to develop.

FIGS. 12–18. Stages in development of archegonium.

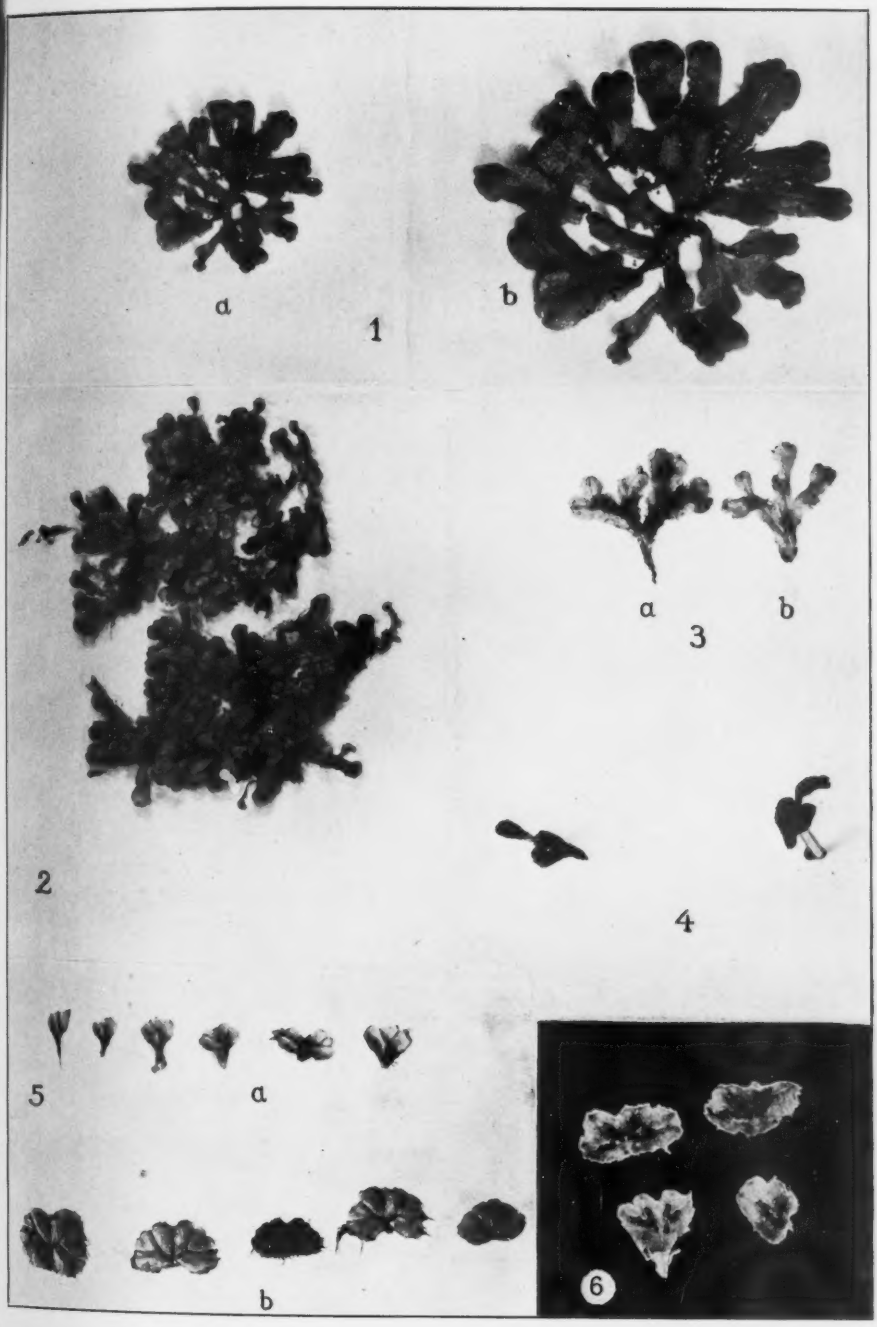
FIG. 19. Archegonium in which egg-cell has not been fertilized and is shrunken.

FIG. 20. Cross-section of neck of archegonium.

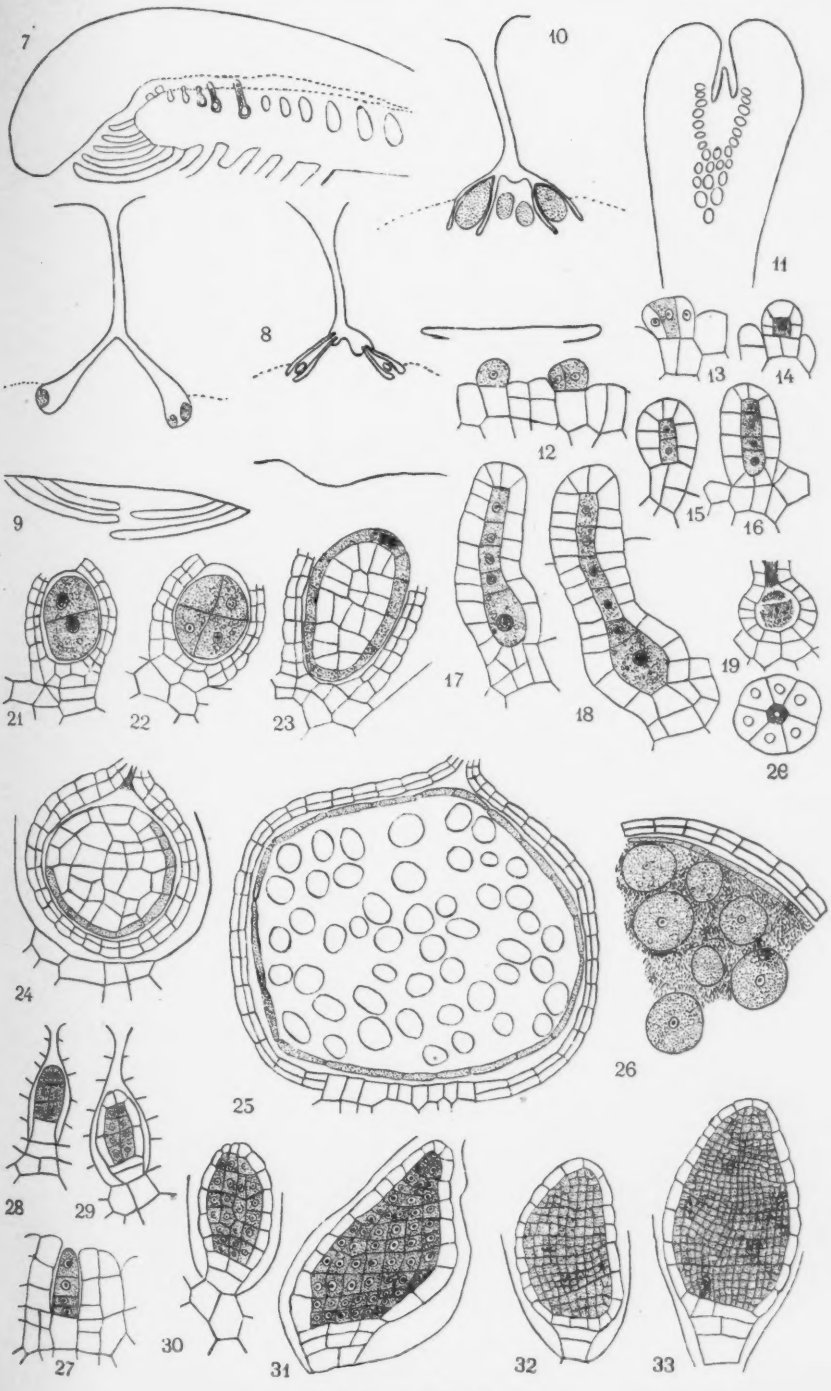
FIG. 21–25. Stages in development of sporophyte.

FIG. 26. Spore mother-cells.

FIGS. 27–33. Development of antheridium. *Figs.* 27–31, from material collected in October.



LEWIS on RICCIA



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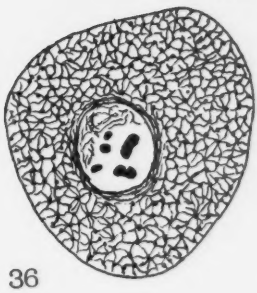

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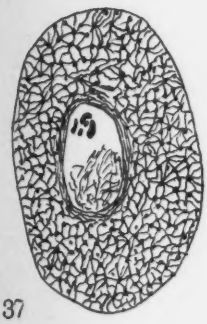

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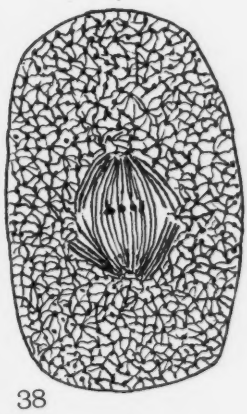

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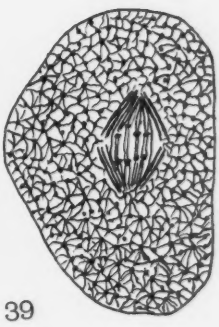

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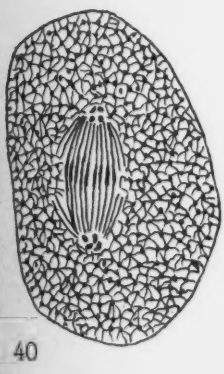

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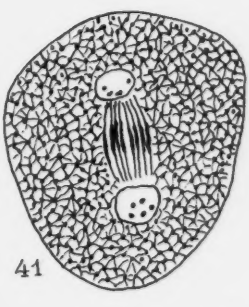

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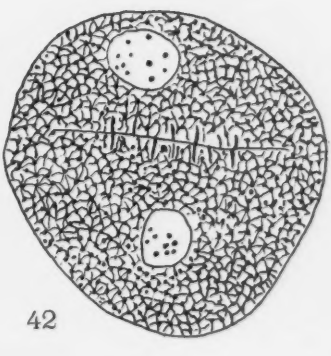

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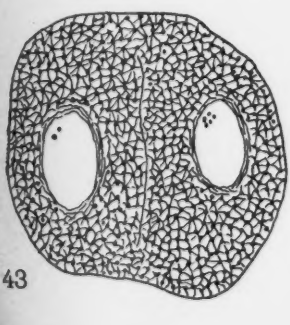

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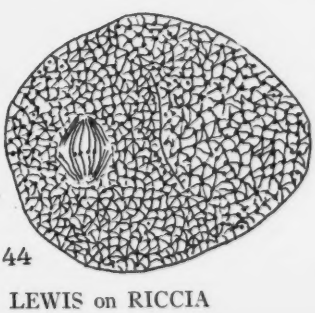

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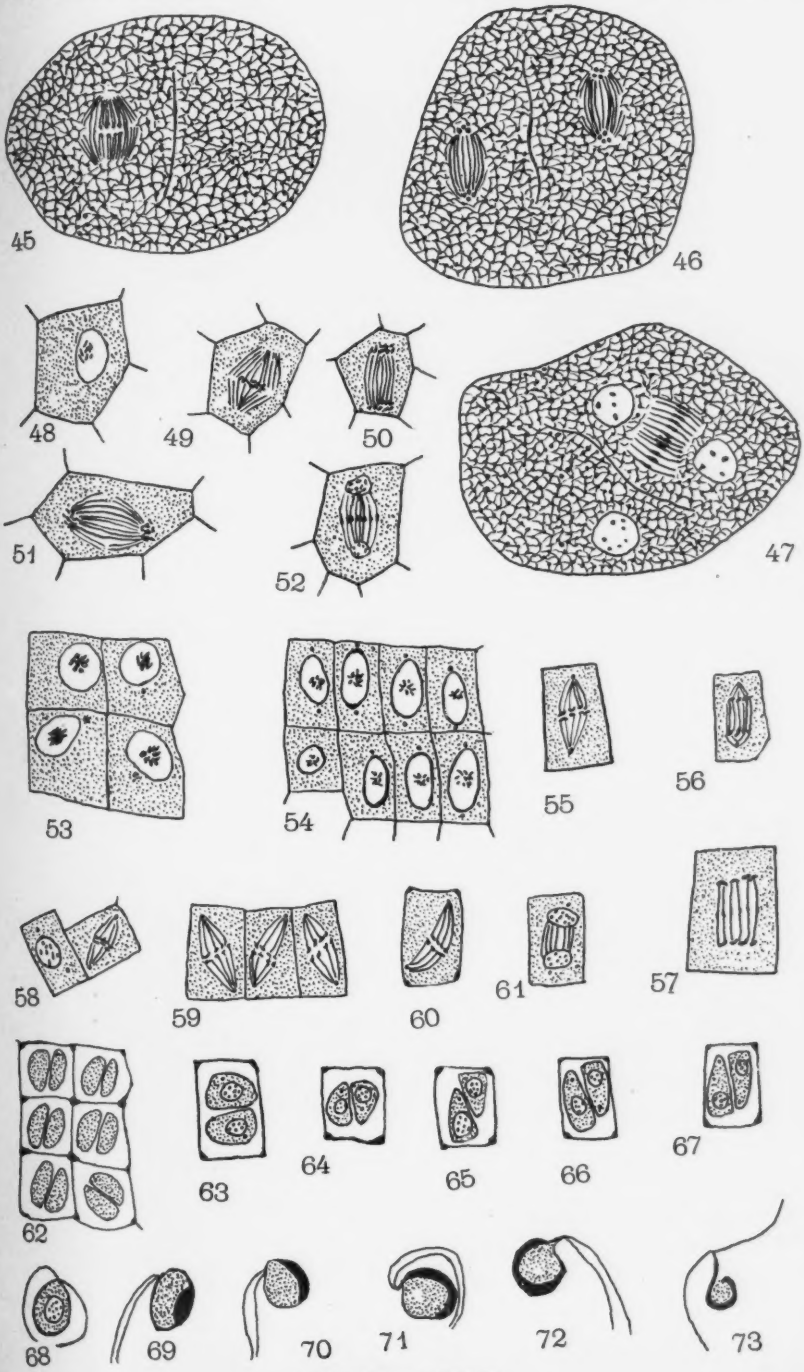

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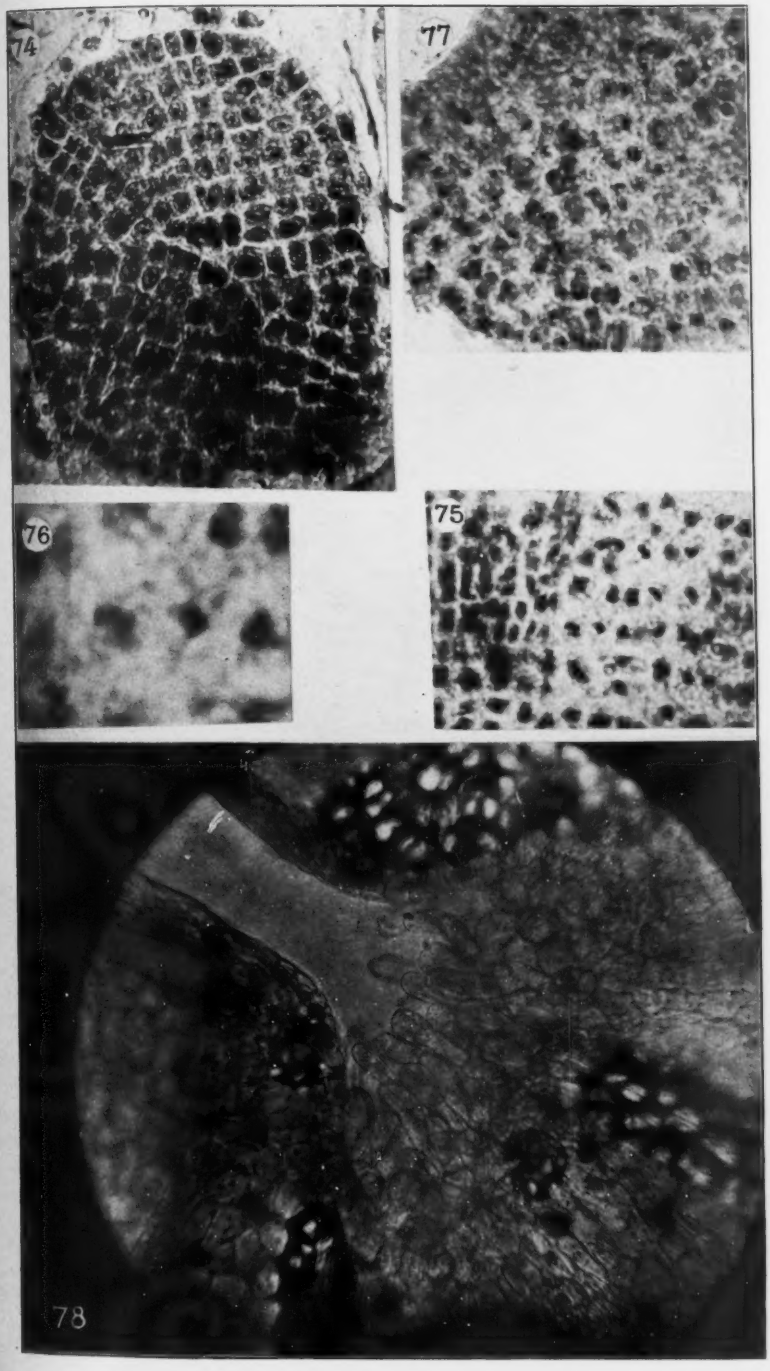

LEWIS on RICCIA

PLATE VII.

FIG. 34. Spore mother-cell in resting state. Chromatin on a fine linin network.

FIG. 35. The chromatin is in the form of an irregular thread.

FIG. 36. Chromosomes formed; weft of delicate fibres about the nucleus.

FIG. 37. Nucleus elongated and showing a weft of fibres.

FIG. 38. Spindle with chromosomes in plate. No centrosome.

FIG. 39. Chromosomes moving to poles of spindle.

FIG. 40. Chromosomes at the poles, thickening of spindle fibres to form cell plate.

FIG. 41. Daughter nuclei.

FIG. 42. Cell plate. Daughter nuclei contain numerous spherical bodies of chromatin which stain bright red with safranin.

FIG. 43. Daughter nuclei preparing for division.

FIG. 44. Daughter nucleus with chromosomes in plate. Neither centrosphere nor centrosome.

PLATE VIII.

FIG. 45. Chromosomes moving to poles.

FIG. 46. Daughter nuclei with chromosomes at the poles to form nuclei of spores. The cell plate formed in the first division persists.

FIG. 47. Second division completed.

FIGS. 48–52. Stages in the division of a sporophyte cell. No centrosome. *Fig. 48* shows slight radiation of cytoplasm from the poles of the elongated nucleus.

FIG. 53. Cells of antheridium which show a single rather irregular body near the nucleus.

FIG. 54. Cells of antheridium which shows the distinct centrosome-like bodies at the poles of the elongated nuclei. Compare *fig. 74*.

FIG. 55. Spindle with centrosome-like bodies at the poles.

FIG. 56. Centrosome-like bodies present when the chromosomes are almost at the poles.

FIG. 57. Cell from young antheridium. Chromosomes at poles. No centrosome can be distinguished.

FIG. 58. One cell preparing for last division, while the adjoining cell has the spindle formed and arranged diagonally.

FIG. 59. Diagonal arrangement of spindles in last division of cells in the antheridium.

FIG. 60. Curved spindles.

FIG. 61. Daughter nuclei formed after diagonal division; centrosome-like bodies present.

FIG. 62. Cells of antheridium after last division.

FIGS. 63–67. Spermatids in mother-cells.

FIGS. 68–73. Stages in the development of the spermatozoids.

PLATE IX.

FIG. 74. Antheridium in which the nuclei are elongated and preparing for division.

FIG. 75. Portion of section of an antheridium showing the spindles with the dense chromosomes in the plate and in some cases the centrosome-like bodies at the poles.

FIG. 76. Two cells of the same section enlarged three times.

FIG. 77. Chromosomes at the poles of the spindles.

FIG. 78. Cross-section of thallus showing the hyaline hairs which extend up into the median grooves.

BRIEFER ARTICLES.

NOTE ON THE RELATION BETWEEN GROWTH OF ROOTS AND OF TOPS IN WHEAT.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXXI.

STUDIES in the experimental morphology of plants have dealt mainly with the subaerial portions, comparatively little attention having been paid to variations in the growth of roots. Thus the literature of the subject is very meager. Several authors, especially MOELLER,[1] DETMER,[2] PERSEKE,[3] MER,[4] GAIN,[5] and FREIDENFELT,[6] have studied the relation of water to the growth of these organs, with the general result that in water itself they grow longer and thinner, with fewer branches and root hairs than are observed in moist soils. Curiously enough, in soils which are too dry for optimum growth, the response is very similar. The roots are long and slender and possess few branches excepting near their tips, which lie, of course, in moister soil.

The only observation on the relation of root growth to that of tops, with which the writer is acquainted, is that of MOELLER, to the effect that in a series of nutrient solutions, of concentrations ranging from 1.0 to 0.05 parts per thousand, the actual weight of roots produced varies generally with the concentration, but that the ratio of weight of roots to that of tops is much larger in the most dilute solution than in any of the others.

A number of experiments have suggested to the author that the accel-

[1]MOELLER, H., Beiträge zur Kenntniss der Verzwergung. Landw. Jahrb. **13**: 167-173. 1884.

[2]DETMER, W., Ueber den Einfluss äusserer Verhältnisse auf die Wurzelentwickelung. Landw. Versuchst. **15**: 107–113. 1872.

[3]PERSEKE, K., Ueber die Formveränderungen der Wurzel in Erde und Wasser. Leipzig. 1877.

[4]MER, E. De l'influence des milieux sur la structure des racines. Comptes Rend. **88**: 1277–1280. 1879.

———Recherches expérimentales sur les conditions de développement des poils radicaux. *Ibid.* 665–668.

———Des modifications de structure et de forme qu'éprouvent les racines suivant les milieux où elles végètent. Assoc. Franc. pour l'avance. sc. Compt. rend. de la 9e session. Rheims. 1880.

[5]GAIN, E., Rôle physiologique de l'eau dans la végétation. Paris. 1895.

[6]FREIDENFELT, F., Studien über die Wurzeln kraütiger Pflanzen. I. Ueber die Formbildung der Wurzel vom biologischen Gesichtspunkte. Flora **91**: 115–208. 1902.

erating or retarding effect of the soil upon plant growth may often be due primarily to a response of the roots themselves, and that the ordinarily observed effect upon the tops may be due to the nature of the roots rather than to that of the soil directly. This question deserves thorough study; the results to be given here cover only a very small portion of the field.

This work was carried on at the laboratories of the Bureau of Soils of the U. S. Department of Agriculture, Washington, D. C. The plant used was the Russian variety of wheat known as "Chul." The plants were grown directly from the seed in paraffined wire baskets of the form described by WHITNEY and CAMERON.[7] As these authors have already pointed out, such baskets possess the advantage over pots of producing a root system uniformly distributed throughout the soil mass, rather than the accumulation of roots on the inner surface of the vessel which occurs in the case of ordinary pots.

The studies to be discussed in this paper were made upon the roots developed in the first six series described in the author's previous publication[8] on the growth of tops, and in similar cultures. The medium used was a very poor soil from Takoma Park, Md., and the same soil with varying amounts of fermented stable manure added thereto.[9] The cultures of any series were placed side by side in a greenhouse, the amount of water in all the baskets being kept practically uniform by weighing at intervals of one or two days and adding the amount of water which was found to have been lost by transpiration. It is thus seen that the different cultures were all subjected to the same conditions excepting those which depend upon the treatment of the soil.

Series I of the paper on growth of tops[10] will serve as an example; the results of all the series are in accurate agreement. The soils and culture numbers were as follows:

Basket Number	1	2	3	4	5	6	7	8
Soil	Takoma Soil untreated	Do.+ 5000 p.p.m.[11] manure	Do.+ 10,000 p.p.m. manure	Do.+ 15,000 p.p.m. manure	Do.+ 20,000 p.p.m. manure	Do.+ 30,000 p.p.m. manure	Do.+ 40,000 p.p.m. manure	Do.+ 50,000 p.p.m. manure

[7] WHITNEY, M. and CAMERON, F. K., Investigations in soil fertility. U. S. Dept. Agric., Bureau of Soils, Bull. 23. 1904.

[8] LIVINGSTON, B. E., Relation of transpiration to growth in wheat. BOT. GAZETTE 40:1 78–105. *figs. 21.* 1905.

[9] For a description of this soil and a discussion of its properties, see LIVINGSTON, B. E., BRITTON, J. C., and REID, F. R., Studies on the properties of a sterile soil. U. S. Dept. Agric., Bureau of Soils, Bull. 28. 1905.

[10] A photograph of the tops and data for their growth are given in that paper.

[11] The abbreviation p. p. m. is used to denote parts per million by weight of air dry soil.

At the end of the experiment, which lasted seventeen days, the soil masses were taken from the baskets and the roots removed from them with as little injury to the latter as possible. The fresh roots thus prepared are shown in *fig. 1*. The numbers correspond to the culture numbers given above. It is at once evident that the root system increases in amount throughout the series. Closer observation shows that this is due mainly to differences in the relative number and length of secondary roots and succeeding branches; the primary roots are of the same number

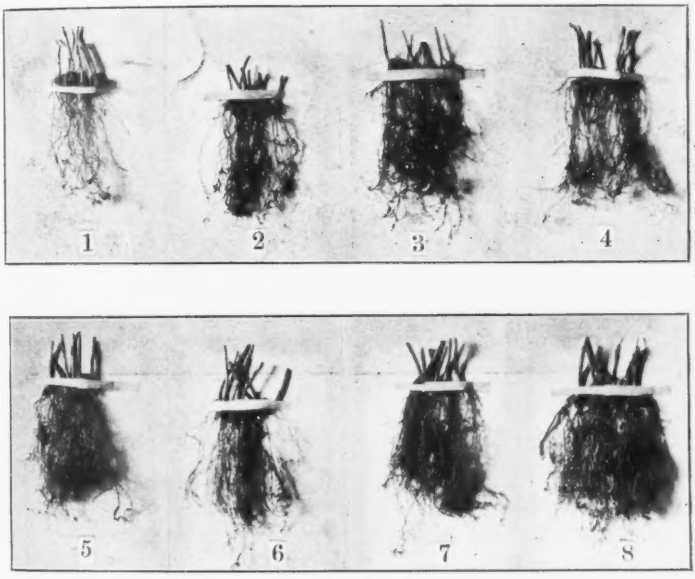

FIG. 1.—Roots from a series of wheat cultures grown in Takoma soil untreated and in the same with addition of 5000 p.p.m. to 50,000 p.p.m. of stable manure.

in culture 8 as in culture 1. They are somewhat longer and more slender in culture 8.

Photographs of single root systems from a similar series are shown in *figs. 2–5*, which bring out the last point more clearly than *fig. 1*. In *fig. 2*, which represents the roots from natural Takoma soil, very few branches are to be seen, and these are exceedingly short; practically the whole root system consists of the primary roots, with a few adventitious roots developed at the extreme base of the stem shortly after germination. *Fig. 3* shows roots from a soil containing 5000 parts per million of manure.

A very slight increase in number and length of branches is to be observed. *Fig. 4*, from a soil containing 10,000 parts per million of manure, shows numerous well-developed branches, while from a soil with 40,000 parts per million of manure, as shown in *fig. 5*, the branches have increased so markedly in number and extent as to make up by far the greater part of the system.

From these facts it is seen that, for this series of soils the variation in growth of tops is correlated with the number and length of lateral roots. The water content of all the soils was the same, so that the variations in

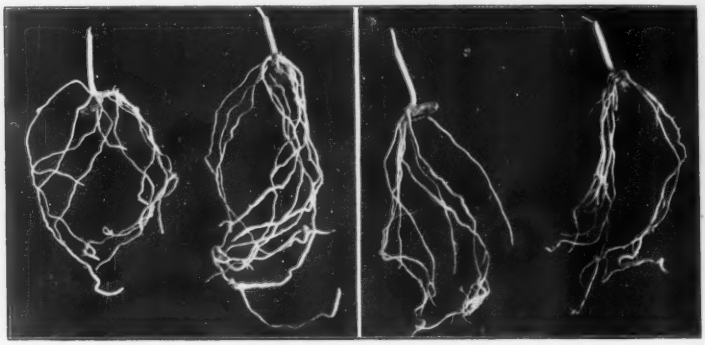

FIG. 2.—Wheat roots grown in Takoma soil untreated.

FIG. 3.—Roots grown in Takoma soil with 5000 p.p.m. manure.

growth cannot be related to this factor; therefore they must be connected with some unknown condition in the soil itself, a condition which is related to the amount of manure present.

The comparative anatomy of these roots was investigated, both by hand and paraffin sections, with the result that in the poor soils the main roots have a strong tendency to swell by direct enlargement of the cortical cells, without increase in the number of these cells, while in the better soils this tendency is not nearly so marked. Very old wheat roots from autumn stubble in the field show this balloon-like enlargement of the cortical cells to a still greater degree. This is apparently a phenomenon of age, suggesting that roots in the poor soils age more rapidly than in the better ones. It was also found that the zone of root hairs, which normally has its lower limit 5–10^{mm} from the root apex, extends in the poor soils to within 1–3^{mm} of the tip. The outgrowth of root hairs from the piliferous layer may also be related to the age of the cells; as is well known, these

organs normally appear only after the cells from which they arise have passed through their period of most rapid growth.

It would seem that the poor soil, by inhibiting branch growth and causing the enlargement of cortical cells, may render the root system unable to carry on an adequate amount of absorption for normal growth, and that this fact may be the main clue to an explanation of the stunted tops in such cases. That the inadequacy of the stunted roots is in regard to the water supply rather than to that of salts, is indicated by the fact that in distilled

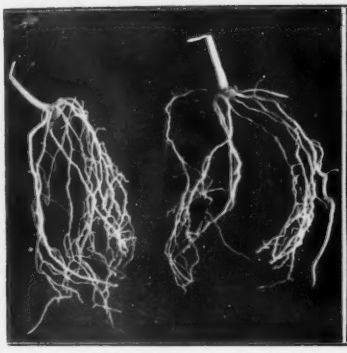

FIG. 4.—Roots grown in Takoma soil with 10,000 p.p.m. manure.

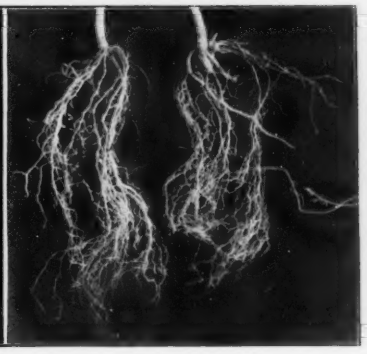

FIG. 5.—Roots grown in Takoma soil with 40,000 p.p.m. manure.

water, for the first two or three weeks, a better growth of tops is obtained than in the natural Takoma soil.

Determination of the relation of the dry weight of the root system to the nature of the soil was deemed advisable, but a number of tests yielded only negative results. The dry weight of the complete system was found to be practically the same from all of the soils. The variations were always irregular. This may, of course, be partially due to the fact that, while it is manifestly impossible to obtain anything like the entire root system of one of the better cultures (owing to the extreme fineness of the branches and their adhesion to the soil particles), yet from the poorer cultures, where branches are short and few, a much larger part of the system is obtainable.—BURTON EDWARD LIVINGSTON, *The University of Chicago, March 5, 1905.*

CURRENT LITERATURE.

BOOK REVIEWS.

The Swiss moors.

FRÜH and SCHRÖTER have published a remarkable work on the moors of Switzerland,[1] and have thus placed all who are interested in bog studies under lasting obligations. More than half of the huge volume is given over to the discussion of general geological, chemical, physical and biological problems connected with peat formation and the ecology of bog plants. One of the most valuable features is the discussion and correlation of the mass of European and foreign literature touching these problems.

After defining the scope of the investigation, the authors take up the peat-producing plant associations of Switzerland. These are described with the greatest detail, from the plankton to the arboreal vegetation; and their relation to peat accumulation is explained.

The moors are distinguished primarily as flat bogs, raised bogs, and bogs of the alpine regions. The first two types are of such general occurrence that their peculiarities may be briefly summarized here.

The *Flachmoor* is characterized as occurring in connection with waters rich in mineral matter, especially lime, in both wet and dry climates. Usually they show centripetal growth, and are dominated by species of Cyperaceae, Gramineae, Juncaceae and Hypneae. Species of Alnus, Betula, and Frangula make up the woody growth. Sphagnum, Ericaceae, and Empetrum are entirely wanting.

On the other hand, the *Hochmoor* type occurs under the influence of waters poor in mineral solutes; where the rainfall is abundant and the temperature mild or cold. The surface is convex. The oldest portion is toward the center; hence the growth is centrifugal. The dominant plants are Sphagnum, Oxycoccus, Andromeda, Calluna, Vaccinium, Empetrum, *Eriophorum vaginatum*, *Pinus montana uncinata*, *Betula pubescens*, and *B. nana*. These plants do not occur on the flat bogs, and are driven out by irrigation with waters of high mineral content, especially lime.

This summary suggests the essential difference between American bogs and the *Flachmoore* of European authors. Grass marshes occur here, which are strictly comparable. But the term "flat bog" is usually applied to areas

[1] FRÜH, J. and SCHRÖTER, C., Die Moore der Schweiz mit Berücksichtigung der gesamten Moorfrage. Beiträge zur Geologie des Schweiz, herausgegeben von der Geol. Komm. der Schweiz. naturf. Gesells. Geotechnische Serie. III Lief. 4to, pp. xviii+751. Bern: A. Francke. 1904. *M* 40.

having a peat substratum, dominated by Sphagnum, Cassandra, Andromeda, Oxycoccus, Ledum, Vaccinium, etc.—a vegetation more nearly related floristically to the *Hochmoor*. Further, these occur in areas whose soil water is rich in mineral salts, frequently overlying marl! The raised bogs of America are strictly comparable to the *Hochmoor* of Europe, but their occurrence appears to be localized by climate rather than by the character of the soil water.

For both the flat and raised-bog plants, the authors conclude that the substratum is physiologically dry because of the combined influence of three factors: (1) high water-content of the substratum, (2) consequent low temperature and (3) the difficulties in the way of root respiration (due to wet soil and scarcity of oxygen), accompanied by a general impairing of all root functions.

The plants of the *Flachmoor* are noted for their ready absorption of mineral salts, absence of mycorhiza, unusual development of the underground parts, and the high percentage of ash. The cause of the xerophytic characters of many of the plants is not clear. They are probably connected with the difficulties of absorption. These plants are strictly hydrophilous, never occurring in dry situations.

The *Hochmoor* plants, however, absorb mineral salts with difficulty; mycorhiza occurs in all the Ericaceae, in Empetrum, Betula, and Pinus; carnivorous plants are common; the root systems are poorly developed; and the ash content is low. The xerophytic character of the plants is in part due to the difficulties of absorption, and in part to their evergreen habit. Many raised-bog plants also occur in dry situations.

The third and fourth chapters discuss the conditions and processes involved in peat accumulation, the chemical and physical properties of the end products, the classification of peats, the bog minerals, and the relation of bogs to coal deposits.

The geographic distribution of the bogs of Switzerland, a geomorphic classification of the moors of the world, the relation of settlement to moor development, the economics of the Swiss moors (with bibliography), the bog deposits as records of the postglacial history of northern Switzerland, form the other principal chapter headings of the first portion of the work.

The second division gives a detailed description of the individual Swiss bogs. In most instances these include not only plant associations of the present surface but also the succession of plant remains occurring in the peat. The location of the bogs is made clear by an excellent topographic map, upon which the distribution of the several bog types is shown. The bibliography occupies seventeen pages and includes only the more important purely scientific papers.

The importance of the work from the standpoint of future investigation is undoubted. To American students it furnishes not only a key to the present status of the subject, but also a model for the study and description of our own bogs, marshes, and swamps.—E. N. TRANSEAU.

Reproduction of mildews.

HARPER has brought together the results of several years of study of nuclear activities in the mildews in a lengthy and beautifully illustrated publication from the Carnegie Institution.[2] It is impossible for us to consider more than the striking new features of his investigations. The paper contains a résumé of much of his earlier work and a broad discussion of many cytological principles which are of general interest and will richly repay the reader of this very creditable contribution to American botany. The author takes a strong stand for critical morphological analysis and classification of the stages in the life history of thallophytes, with a clear separation of phylogenetic history from physiological functions.

The most important new features of HARPER'S research, chiefly in Phyllactinia, are (1) the establishment of a "central body" within the nucleus, which constitutes a point of attachment for the chromatic elements and gives a clear polarity to the structure, and its continuous existence through the most important phases in the life history; (2) the evidence for the permanence of the chromosomes; and (3) the evidence that the triple mitoses preceded by synapsis in the ascus constitute a double reduction of the chromosomes which are quadrupled by the two nuclear fusions in the life history, the first fusion at the time of the sexual act and the second fusion within the young ascus.

The central body is a permanent structure, always present in the resting nucleus, dividing with each mitosis, and the center for an arrangement of chromatic threads within the nucleus and for the attachment of spindle fibers during nuclear division. Its position determines a pole in the nucleus around which are grouped the chromatic elements, which are thus always in connection with the central body, both in the resting nucleus and during mitosis. This constitutes new evidence for the permanence of the chromosomes throughout the succession of mitoses in the life-history. HARPER has not been able to distinguish the different sets of chromosomes after the nuclear fusions, for the chromatic elements and the central bodies unite very intimately. But the second fusion in the life history, that in the ascus, is followed at once by a period of synapsis and the triple mitoses out of which come the eight chromosomes characteristic of the gametophytic phase of the form.—B. M. DAVIS.

MINOR NOTICES.

Observations in Spitzbergen.—The flora of Spitzbergen is fairly known. Therefore, DR. WULFF, who accompanied the Swedish expedition for the measurement of an arc of the meridian, undertook to make ecological observations on the arctic plants,[3] especially touching their transpiration, occurrence of mycorhiza

[2] HARPER, R. A., Sexual reproduction and the organization of the nucleus in certain mildews. Imp. 8vo. pp. 104. *pls.* 7. Washington: Carnegie Institution of Washington. 1905.

[3] WULFF, THORILD, Observations botaniques faites au Spitzberg. Missions scientifique pour la mesure d'un arc de méridien au Spitzberg. Mission Suédoise. Tome II, X[e] section, Botanique. Traduit de l'Allemand par H. MARCEL HARDY à Dundee. 4to. pp. 63, *pls.* 4. Stockholm. 1903.

and anthocyan, the vegetation of the "polygonal" soils, and to make miscellaneous floristic notes at various stations. The transpiration he finds very feeble and almost without diurnal periodicity or plant control. This feeble transpiration he accuses of being a *cause* of feeble growth; instead, is not its feebleness due to the same cause as the feebleness of growth, the low supply of energy? Mycorhizas, internal and external, are common. Anthocyan is found in fifty species, about half the known higher plants. It is always lacking in plants growing on soil enriched by the droppings of wild birds, whereas the same species growing on poor soils show it abundantly. As to the rôle of anthocyan, he holds it for an absorber of energy, and without it no plant can become dominant in arctic regions. For other interesting observations one must consult the work itself.—C. R. B.

Polypodiaceæ and edible fungi.—Not that there is any connection between them; but both are treated by COPELAND in a bulletin[4] from the Government Laboratories at Manila. The section on Polypodiaceæ forms the bulk of the bulletin and is "an attempt to collect and publish descriptions of all the ferns known to have been found in these islands." The author adds: "I am not personally acquainted with a large part of those ferns still known here only from earlier collections." Which leads us to remark that he should then have abstained from describing a new genus and new species among them, as he did in DR. PERKINS's last *Fragmenta*. In reprinting here these descriptions he has neglected to indicate that they have already been published elsewhere. He has sinned again in adding one more new name in this bulletin. The compilation of such descriptive floras is undoubtedly serviceable; but one who is not a taxonomist and who confesses the absence of indispensable books and specimens, should not take the chances of cumbering pteridology with new names which may or may not be justified. And the same may be said regarding the brief fungus part.—C. R. B.

Genera of Mexican plants.—The flora of Mexico is so closely related to our own that any work on it is of essential assistance to American taxonomists. So we welcome the assembling and description of the Mexican genera, and the listing of the species, undertaken by Professor CONZATTI, director of the State Normal School of Oaxaca, of which the first volume, on Polypetalae, has recently been published by the Ministry of Public Works. This volume[5] begins with an artificial key covering about 50 pages including all genera, and contains descriptions of 667 genera of Polypetalæ, representing 71 families, and including close to 4,500 species. This is to be followed by another on Gamopetalae and a third

[4]COPELAND, E. B., I. The Polypodiaceæ of the Philippine Islands. II. New species of edible Philippine fungi. Bureau of Government Labs. Bull. 28. 8vo. pp. 146. *pls.* 3. 1905.

[5]CONZATTI, C., Los géneros vegetales mexicanos. Imp. 8vo. pp. 449. Mexico: Oficina Tip. de la Secretaría de Fomento. 1905. $3 (Mexican).

on Monochlamydeae, Monocotyledoneae, Gymnospermeae, and Pteridophyta, embracing in all about 1900 genera.

The descriptions are very full, and though the diagnostic characters are not indicated, this is largely atoned for by the complete system of synoptic characters under the tribes and subtribes.—C. R. B.

Germs of mind in plants.—A little book,[6] unknown to us in the original French, now translated into English by A. M. SIMONS, well-known for his work in Chicago along social and philanthropic lines, shows that there exists in France the same sort of popularizers of science as in our country—writers who with a smattering of scientific knowledge lack the fuller knowledge that forms a background and furnishes scientific perspective. The facts of plant ecology are herein so distorted in their relation as to become caricatures; the use of words is so fanciful as to convert sober ideas into grotesque fairy-tales. For this, doubtless, the author is chiefly responsible; but the translator slips occasionally through unfamiliarity with a technical use of some common word.

The book is interesting; but it is as little "science" as a historical novel is history. It is difficult to see how such fiction can be "a contribution to the cause of socialism and science."—C. R. B.

Hepaticae of France.—LACOUTURE has prepared a helpful series of descriptive analytical keys to facilitate the identification of French liverworts by amateurs.[7] The keys are arranged in a convenient bracket fashion, which is easy to use but makes the form of the thin volume rather unhandy and precludes its use as a field manual. The description of each species is accompanied by an excellent figure illustrating the most essential features described. The keys, in the form of tables, are arranged in three series, of which the first, consisting of tables I and II gives the characters of the tribes; the second, tables III–IX the characters of the genera; and the third, tables XII–XXXIX, the characters of the species and the illustrations. No attempt is made to exhibit the natural classification.—C. J. CHAMBERLAIN.

Index Filicum.—The fourth and fifth fascicles of CHRISTENSEN's important work[8] were issued respectively in October and December last. They carry the references from *Cyathea lanuginosa* to *Gleichenia cryptocarpa*. The huge genus Dryopteris alone takes fifty-two pages, which indicates something of the comprehensiveness of the work. Let colleges and libraries hasten to support by their

[6] FRANCÉ, R. H., Germs of mind in plants. Trans. by A. M. SIMONS. 12mo. pp. 151. Chicago: C. H. Kerr & Co., 1905. 50 cts.

[7] LACOUTURE, CH., Hépatiques de la France. Tableaux synoptiques de caractères saillants des tribus, des genres, et des espèces. 4to. pp. 78. *figs.* 200. Paris: Paul Klincksieck. 1905. *fr.* 10.

[8] CHRISTENSEN, C., Index Filicum, etc. Fasc. 4, 5. Copenhagen: H. Hagerups Boghandel. 1905. Each *3sh. 6d.*

orders the stupendous and too thankless task which the author has undertaken. The employment of the American system of citation is notable.—C. R. B.

Das Pflanzenreich.[9]—Of this work parts 22 and 23 have lately appeared, including respectively the Primulaceae by PAX and KNUTH, and the Halorrhagaceae by SCHINDLER. The rate at which these monographs are appearing is remarkable, and shows something of the energy of the editor and his sagacity in the selection of his collaborators. The publisher's part, too, is admirably done.—C. R. B.

Eucalyptus.—MAIDEN'S revision[10] has now reached part 7, which includes *EE. regnans*, *vitellina*, *vitrea*, *dives*, *Andrewsi*, and *diversifolia*, and is illustrated by four plates.—C. R. B.

NOTES FOR STUDENTS.

Items of taxonomic interest.—ZAHLBRÜCKNER lists (Beihefte Bot. Cent. **19**[2]: 75–84. 1905) the lichens collected by Professor D. H. MEYER in the Ecuador highlands in 1903, describing six new species.—CARDOT (*idem* 85–148. *figs. 39*) enumerates 125 species of the mosses of Formosa, collected by Abbé FAURIE in 1903, bringing the total known species of this island to 130, of which 39 are new. *Herpetineuron* (C. Müll. as Anomodon §) is raised to generic rank.—ENGLER describes (Bot. Jahrb. Syst. **37**: 95, 96. 1905) a new genus of Araceae, *Ulearum*, and in his tenth contribution to a knowledge of the Araceae, (*idem*) adds to the family nearly a hundred new species, chiefly from Central America, the subequatorial andine province, the Philippines, and East Indies.—DIETEL, in his sixth paper on Japanese Uredineae (*idem* 97–109) describes 16 new species, and in one on Japanese fungi (*idem* 156–160) ten others.—RADLKOFER (*idem* 144–155) describes 8 new species of Serjania and 8 of Paullinia (Sapindaceae) from Peru, Brazil, Bolivia, and Columbia.—STEPHANI (Bull. Herb. Boiss. II. **5**: 885–900, 917–946. 1905) in his *Species Hepaticarum* concludes the treatment of the genus Plagiochila, describing 26 new species, a number of them from equatorial America.—DOMIN (*idem* 947, 948) describes 2 new species of Koeleria from Asia, and BEAUVERD (*idem* 948) a new Burmannia from Brazil and (990–991) a new Hesperantha from the Transvaal.—FERNALD characterizes (Ottawa Nat. **19**: 156. 1905) a new variety of *Antennaria neodioica* Green from E. Quebec.—SCHNEIDER, in a prodromus to a monograph of Berberis (Bull. Herb. Boiss. II. **5**: 139 ff. 1905) recognizes 159 species, among them a number of new ones of his own creation, which he divides into 21 sections. The regions of their

[9] ENGLER, A., Das Pflanzenreich. Heft 22, Primulaceae by F. PAX and R. KNUTH. pp. 386, *figs. 75* (*311*), *maps 2*. *M* 19. 20.—Heft 23. Halorrhagaceae by ANTON K. SCHINDLER. pp. 133, *figs. 36* (*196*). *M* 6. 80. Leipzig: Wilhelm Engelmann. 1905.

[10] MAIDEN, J. H., A critical revision of the genus Eucalyptus. 4to. pp. 183–205, *pls. 33–36*. Sydney: Government N. S. Wales. 1905. *2sh. 6d.*

dominance are South America and E. Asia.—HELLER describes (Muhlenb. 1: 124) a new Veratrum from Idaho, and (*idem* 125) a Linanthus or Gilia from California.—MCALPINE adds a new genus, *Uromycladium*, to the Uredineae (Ann. Mycol. 3: 303–323. *pls. 6–9.* 1905). It is based on 7 Australian species occurring on Acacias, and is intermediate between Uromyces and Ravenelia.—VUILLEMIN shows the identity of *Hartigiella* with *Meria* (*idem* 340–343).—SCHMIDLE found in plankton material from Roxheim, Bavaria, a new alga, for which (Hedw. 45: 34. 1905) he erects the genus *Didymogenes*.—FLEISCHER (*idem* 53 ff.) proposes an extension of the moss family Pterobryaceae to include five other families, in whole or in part, and gathers from various genera some 25 species to swell his genus Pterobryopsis. He establishes a new monotypic genus *Müllerobryum* on an Australian moss already referred to 3 separate genera. *Trachypodaceae* is a new family, and *Trachypodopsis* its characteristic new genus, for both of which he has "gathered of every kind," and Teil I is only begun!—PECK (Rept. N. Y. State Botanist **1904**) describes new fungi; Boletus (3), Clavaria (2), Cortinarius, Lactarius (2), Pholiota.—CARDOT finds 35 new species of mosses in SKOTTSBERG'S collections made on the Swedish antarctic expedition (Bull. Herb. Boiss. II. 5: 997–1011. 1905).—HIERONYMUS has studied (Bot. Jahrb. Syst. 36: 458–573. 1905) the Compositae collected by JELSKI in Peru, among which he finds 58 new species.—DIELS (*idem* Beiblatt 82: 1–138) makes hundreds of additions to his flora of central China, including many new species and three new genera, *Giraldiella* Dammer (Liliaceae), *Pteroxygonum* Dammer and Diels (Polygonaceae), and *Biondia* Schlechter (Asclepiadaceae).—NELSON describes (Proc. Biol. Soc. Wash. 18: 171–776. 1905) new species from Nevada in Cleomella (2), Sphaerostigma, Zauschneria, Rhamnus, Polemonium, Artemisia, and a new genus of Solanaceae, *Bosleria*.—RENAULD and CARDOT in their tenth paper on *Musci Exotici* (Bull. Soc. Roy. Bot. Belgique 41: 7–122. 1905) describe, among many others, largely Mascarene and East Indian, 9 new species from Porto Rico, 3 from Costa Rico, one from Guadeloupe, 3 from Cocos Island (Pacific Cent. Am.), 1 from Mexico, and 1 from Hawaii. They also establish as a new genus of Hypnaceae Müller's section of Hypnum, *Dimorphella*. The same authors (*idem* 123 ff.) in their third article on *Musci Costaricenses* describe 22 new species.—HELLER has found some new species in his collections for 1905 in California and describes them (Muhlenb. 2: 1–6. 1905), under Eriogonum (3), Montia, Delphinium, Ranunculus, Thysanocarpus (2), Lithophragma, Ribes, and Amelanchier.—HOWE adds several algae to our flora (Bull. Torr. Bot. Club 32: 563–586. *pls. 23–29.* 1905) from the Bahama-Florida region; Halimeda, Avrainvillea, Sarcomenia, Dudresnaya, and a new genus *Cladocephalus* (Codiaceae), besides changing several names.—UNDERWOOD (*idem* 587–596) maintains the genus *Alcicornium* Gaud. as valid, gives a synopsis of the species, and describes *A. Veitchii* as a new species.—RYDBERG, about to publish a Flora of Colorado, makes (*idem* 597–610) what he considers necessary changes in names, and describes new species of Deschampsia, Eatonia, Poa (9), Festuca (2), and

Elymus (2).—OSTERHOUT proposes from Colorado (*idem* 611–613) new species of Allionia, Aster, Senecio, and Carduus (2), which are respectfully referred to Mr. RYDBERG.—SARGENT adds (Rhodora 7: 192–219. 1905) 24 new species of Crataegus, all from New England.—ROBINSON describes (*idem* 219–222) a new Ranunculus from Gaspé and Labrador.—C. R. B.

Fossil gymnosperms.—Two trunks of Cycadoidea have been found in the Portland beds of Boulogne, to which MM. FLICHE and ZEILLER give the specific name *C. pumila* on account of their small size.[11] Another Cycadoidea is described without attribution of a specific name. An interesting and important discovery is a cone of Sequoia of the *S. gigantea* type, which is named *S. portlandica*. The oldest well authenticated cone of Sequoia previously known is Heer's *S. lusitanica* from the Wealden beds of Portugal, which belongs to the type represented by the living *S. sempervirens*. It thus is demonstrated that Sequoia existed in its two living types as far back as the Jurassic period and must thus be very much more ancient in its first appearance. Other important discoveries are pine-cones representing the two main series of the present day, viz., the sections Strobus and Pinaster. The cone of the Strobus type is very much flattened and does not yield any definite information as to its internal organization, so the authors include it under the provisional fossil genus Pinites, with the specific appellation *P. strobiformis*, which would appear to be too close to our western *Pinus strobiformis* to stand as a permanent name. The other cone is exceedingly well preserved and resembles very closely, as the authors point out, small cones of the living *P. Laricio*. This cone is referred to Pinus as *P. Sauvagei*. These observations are of very special interest because they establish that Pinus too must be a very old genus, since examples of both the hard and soft pine series existed already in the Jurassic.

GOTHAN calls attention to the somewhat unsatisfactory condition of *Xylopalaeontolgie* at the present time and by comprehensive study of fossil and living woods, including many type-specimens of the former, reaches a number of conclusions of greater or less importance.[12] The proposition of FELIX to divide fossil woods presenting tracheary structure resembling that of living Araucarineae, into Cordaioxyla for the palaeozoic woods, which may be supposed to be those of Cordaites, and into Araucarioxyla for mesozoic and later woods, is rejected, since in the author's opinion no distinction can be made histologically between the two. For these woods ENDLICHER'S name *Dadoxylon* is retained. *Cedroxylon* Kraus and *Cupressinoxylon* Goeppert are separated from each other, not on the basis of the presence of resiniferous parenchyma in the latter genus and ts absence in the former, but on the character of the medullary ray-cells, since many Cedroxyla and even Pityoxyla have resinous parenchyma. This distinction has

[11] FLICHE, P., et ZEILLER, R., Note sur un florule portlandienne des environs de Boulogne-sur-Mer. Bull. Soc. Geol. de la France IV. 4: 787–812. 1904.

[12] GOTHAN, W., Zur Anatomie lebender und fossiler Gymnospermenhölzer. Abhandl. k. preuss. geol. Landesanstalt, Neue Folge, Heft 44. 1905.

already and previously been clearly made by PENHALLOW. The author also attempts to separate the woods of the Podocarpeæ from those of the Cupressineae in the larger sense, on the basis of the structure of the pits in the ray-cells. The success of this distinction may be judged from the fact that it results in putting Sciadopitys with the Podocarpeae. *Pityoxylon* of Kraus is broken up by this writer into two genera, *Piceoxylon* and *Pinusoxylon*. The latter genus represents the wood of Pinus, and seems somewhat unfortunate, since it is doubtful if the mesozoic pines had the wood structure which is found as characteristic of that genus in Tertiary and modern times. There are also disquisitions on spiral striation in the wood of the gymnosperms and on the value of annual woody rings as diagnostic of geologic formations. The work closes with two tables for the determination respectively of living and fossil gymnospermous woods. There is likewise an index and an alphabetical list of the living woods investigated by the author.—E. C. JEFFREY.

Injury by smoke.—Frequent controversies and law suits, arising from damage to agricultural crops by the smoke produced by manufacturing establishments in Germany, have made the recognition of this form of injury extremely important. In order to furnish a basis for distinguishing smoke-injury from injuries due to other factors, SORAUER[13] has made a comparative anatomical study of various kinds of injury commonly occurring in the more important grains, oats, wheat, and barley. The paper contains detailed comparative descriptions of changes in the cell walls and cell contents which cannot be severally noted here. The general plan followed in each case is represented by the following heads: The behavior of the normal plant in its gradual, natural dissolution; abnormalities in smoke-free regions; the phenomena in plants injured by chlorin and by hydrochloric acid fumes; experimental tests of the influence of hydrochloric acid fumes; phenomena confused with smoke injuries. In natural death the cells lose a large part of their contents and finally (except the epidermal cells) collapse completely. This process first involves the tip and edges of the leaves. In cases of death resulting from other causes, as drought, the cells do not collapse so completely, since the contents are not fully resorbed. In injuries due to acid fumes from smoke, the contents of the mesophyll cells contract into an irregular greenish lump, while the cell walls partially collapse.

The most striking feature about this form of injury is the collapse of the epidermal cells. The accompanying changes of the cell contents and cell walls in these and in many other forms of injury are minutely described. The recognition of smoke injury in general is based on the fact that the cells, dying rapidly, collapse partially without being emptied of their contents, the epidermal cells showing the same phenomena. The author continually emphasizes the fact, however, that no clearly defined symptoms for the absolute and certain recognition of smoke injury can be given, but that in all cases a comparative study of

[13] SORAUER, P., Beitrag zur anatomischen Analyse rauchbeschädigter Pflanzen. Landw. Jahrb. **33**:585–664. *pl. 15–18.* 1904.

plants growing under the immediate influence of the acid fumes and others growing under similar conditions but not within the smoke zone, must be made. —H. HASSELBRING.

Viticulture.—Recent publications from the Royal Hungarian Central Institute of Viticulture are as follows: Volume III, part 2, consists of chemical analyses of the stems and shoots of American species used for stocks in Hungary.[14] The points determined were the moisture content, ether extractives (oils, fats, waxes, gums, and organic acids not further determined), alcoholic extractives (tannin, glucotannin, vanillin, and organic acids), nitrogen, starch, cellulose, and proteids. The paper contains a large number of analyses made at different seasons, but no general results have yet been reached, and it is difficult to see what may be expected. Part 3 of this volume is a small paper by ISTVÁNFFI[15] in which he describes a disease of the vine caused by *Phyllosticta Bizzozeriana* Massal. The disease is not of great importance, but has been mistaken for the black rot, one of the most dangerous vine diseases. In the part 4 ISTVÁNFFI[16] gives the results of his investigations on the gray rot, caused by *Botrytis cinerea*. The first part of this paper is taken up with the effects of various kinds of poisons and other treatments as cold, drying, etc., on the spores of the fungus. One of the most striking results is the unusually high resistance which the spores are said to have to copper. Spores were kept twenty-four hours in different strengths of Bordeaux mixture ranging from 1 to 10 per cent., to which was then added must containing 1 per cent. of tartaric acid, so that the resulting solutions contained the equivalent of 0.3 per cent. $CuSO_4$. Of the spores from the lowest strength mixture 38–40 per cent. germinated, of those in the highest 10–12 per cent. germinated. Spores sown on berries in 3 per cent. Bordeaux mixture germinated and penetrated the epidermis. Spores, kept one hour in a 2 per cent. solution of $CuSO_4$, which was then diluted with ten times its volume of must, germinated. Many other similar experiments are given. The second part of the paper deals with the development and life history of *Botrytis cinerea* and methods of control. Very little new is added to the life history of the fungus. For treatment, spraying with a 5 per cent. solution of calcium bisulfid is recommended.—H. HASSELBRING.

Endotrophic mycorhiza.—The long and important paper of GALLAUD[17] on this subject merits brief summary, as his conclusions are quite revolutionary. He has described for the first time the anatomical and cytological characters of

[14] GASPAR, J., Analyses des sarments américains. Ann. Inst. Cent. Ampél. Roy. Hongrois **3**:57–166. *pls. 4–12.* 1905.

[15] ISTVÁNFFI, Gy. de, D'une maladie de la vigne causée par le *Phyllosticta Bizzozeriana*. *Idem*, 167–182. *pl. 13.* 1905.

[16] ISTVÁNFFI, Gy. de, Études microbiologiques et mycologiques sur le rot gris de la vigne. *Idem*, 183–360. *pls. 14–21.* 1905.

[17] GALLAUD, I., Études sur les mycorhizes endotrophes. Rev. Gén. Bot. **17**: *pls. 4.* 1905.

a large number of endophytes, and his study enables him to distinguish four types: (1) type of *Arum maculatum*, hyphae intercellular after traversing the outer cells, their growth arrested by formation of simple terminal haustoria which penetrate the cortical cells; (2) type of *Paris quadrifolia*, hyphae intracellular, of indefinite growth, with complex lateral haustoria arising at definite points; (3) type of Hepaticae, hyphae intracellular, of indefinite growth, entering *via* rhizoids and bearing haustoria transformed into sporangioles; (4) type of Orchideae, hyphae intracellular, of indefinite growth, forming tight pellets which are sometimes permanent and sometimes undergo more or less complete digestion.

There is a remarkable uniformity in the constitution of the cell walls and in the cytological structure. Repeated attempts to isolate the fungi by direct extraction and by inoculation were unsuccessful. The first method failed, probably because the fungus already in was already too much altered by the digestive action of the host, and the second leads the author to distrust utterly the identifications of previous authors. The endophytes, he holds, are *saprophytes internes*, which by their highly differentiated haustoria borrow some non-living nutritive material from the cells in which they live. These cells react very rapidly on the fungus, killing its haustoria, digesting and absorbing them in part; then they resume their normal life, momentarily disturbed. It cannot be said that there is a harmonious symbiosis between the two plants, but rather a conflict between the invading, but little harmful, fungus and the cells which defend themselves by their digestive power.—C. R. B.

Sexual reproduction of Stigeoclonium.—PASCHER in an account of the sexual reproduction of *Stigeoclonium fasciculatum*,[18] touches briefly on the formation and behavior of the zoospores (macrospores), which in general agrees with that of other forms, but in a few cases the sporelings developed into filaments of a few cells only, which then formed in each cell a single four-ciliate zoospore (macrospore) that developed like other zoospores. The microspores are four-ciliate and long motile; after losing their motility they become spherical and either form resting-cells, or (rarely) conjugate and form zygotes. The development of the latter was not followed, but from hasty observation he concludes that their germination does not depart from that of the zoospores or the resting-cells. After an indefinite period the resting-cells germinate like the zoospores. Some, however, (akinetes or palmella stage), grow into a few-celled filament, each cell giving rise to four biciliate zoospores, resembling the microspores in size and activity, except that they will not conjugate but germinate at once like the zoospores.

Phylogenetically he claims for *Stigeoclonium fasciculatum* a position midway between Ulothrix and Draparnaldia, the three kinds of spores indicating that it is on the border-line of sexual reproduction. The same position was long ago claimed by DODEL-PORT for *Ulothrix zonata*. But such generalizations will bear

[18] PASCHER, A., Zur Kenntnis der geschlechtlichen Fortpflanzung bei Stigeoclonium. Flora **95**: 95–107. *figs. 2.* 1905.

revision, and investigations of the cytological phenomena involved are especially needed. PASCHER'S observations were microscopic to be sure, but he has apparently attempted no cytological observations at all.—R. THIESSEN.

Sigillarian stems.—Owing to the rarity of sigillarian stems showing structure the description of new specimens is of particular interest to paleobotanists. KIDSTON[19] has given a well-illustrated and adequate description of *Sigillaria elegans*, which differs from the historic *S. Menardi* in that the primary wood of the former is continuous instead of broken up into bundles. The protoxylem is external to the metaxylem, and both are composed of scalariform tracheids. The secondary wood is about equal in thickness to the primary, and shows medullary rays which are mostly one cell thick and one to nine cells high. The outer margin of the primary wood is crenate, and from the furrows arise the leaf traces, of which there are about twenty-eight in a cross section; these do not seem to possess any secondary wood. As is usual in sigillarian stems the pith, phloem, and inner cortex have perished, and the outer cortex contains a broad zone of periderm. *S. elegans*, with a continuous ring of primary xylem, *S. spinulosa*, with a mixture of continuous and discrete xylem, and *S. Menardi*, with separate bundles, form a good series, and judging from the scanty data available it seems that this series represents a sequence in time. The features of *S. elegans* support the view that the genus sprung from forms more like Lepidodendron.—M. A. CHRYSLER.

Mycoplasmic propagation of grain rust.—ERIKSSON has published another instalment of his studies on the demonstration of the propagation of grain rust by means of mycoplasm, this time dealing with *Puccinia graminis*.[20] Four means are recognized by which the uredo stage of the rust may possibly arise in spring time in winter wheat: (1) from spores of the barberry aecidium, which in turn arose from the resting teleutospores that had remained dormant over winter; (2) direct infection of the wheat plant from the resting teleutospores (homoecism); (3) uredo infection from mycelium remaining alive in the wheat plant over winter; and (4) from endogenous germs of disease (mycoplasm) which pass the winter in a resting condition in the live wheat plant. He marshals a large array of data, drawn from his own observations and experiments and from a wide range of literature, to show that the first method, although it exists, is by no means universal, that the second is highly probable, that the third never occurs in northern regions, if anywhere, and that the fourth is the most common method everywhere. Although the conclusions of the author will not be accepted by most investigators of this difficult problem, yet the array of data is interesting. Two clearly drawn

[19] KIDSTON, ROBERT, On the internal structure of *Sigillaria elegans* of Brongniart's *Histoire des végétaux fossiles*. Trans. Royal Soc. Edinburgh **41**:533–550. *pls. 1–3*. 1905.

[20] ERIKSSON, JAKOB, Ueber das vegetative Leben der Getreiderostpilze IV: *Puccinia graminis* Pers. in der heranwachsenden Getreidepflanze. Kungl. Sv. Vet.-Akad. Handl. **39**[5]:1–41. *pls. 1, 2*. 1905.

colored plates are used to show the author's interpretation of the transformation of the resting mycoplasm into the mycelium condition of the rust.—J. C. ARTHUR.

Light relations at high altitudes.—WIESNER'S study of the *Lichtgenuss* of plants, already comprehensive for varying latitudes, has now been extended[21] to include high altitudes. During a period of thirty days from Aug. 16, photometric observations were made in the Yellowstone territory at eight altitudes ranging from 515 to 2210^{m} above sea level. The investigation shows that the behavior of plants with advancing latitude does not agree with that manifested under increasing altitude. The relative amount of available light appropriated by arctic plants increases inversely with the distance from the pole. This relation holds with increasing altitude only to a certain limit, above which a smaller and smaller share of available light is appropriated. The cypress habit of growth is evidently intended to protect from increased intensity of light, whether this accompanies low latitudes or high altitudes. This seems all the more probable because in such altitudes species having this habit do not show a defoliation from heat, which is manifested by other species that do not show it at lower levels.—RAYMOND H. POND.

Tomato rot.—VON OVEN[22] has recently described a disease of tomatoes caused by *Fusarium rubescens* Appel & Von Oven. This fungus causes a rotting of the tomato fruit, and evidently does not belong to the fungi in this group producing stem rot or wilt disease, although in cultures the pink and violet shades characteristic of the latter are also produced by this new species. As it is impossible to separate the species of Fusarium on morphological grounds, VON OVEN has attempted to distinguish this species at least from several disease-producing fusariums by their physiological characteristics. It is thus distinguished from *F. Solani*, *F. putrefaciens*, and *F. rhizogenum*. In cultures on sterilized potato small sclerotia were formed, which produced conidia after being exposed during December and January. The author concludes that this is a hibernating stage of the fungus, although he does not mention finding them in nature.—H. HASSELBRING.

Axillary scales of aquatic monocots.—As aquatic monocotyledons are by some held to be modern representatives of the more primitive angiosperms; as these forms may have been genetically related to some such type as Isoetes; and as he regards the ligule as an important phylogenetic organ, GIBSON[23] has made a study of the vestigial structures of the following families: Potamogetonaceae,

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[21] WIESNER, J., Untersuchungen über den Lichtgenuss der Pflanzen im Yellowstonegebiete und in anderen Gegenden Nordamerikas. Sitzungsber. Kaiserl. Akad. Wiss. Wien, Math.-Naturw. Klasse **114**[1]:(pp. 74.) *figs. 2.* 1905.

[22] OVEN, E. VON, Ueber eine Fusariumerkrankung der Tomaten. Landw. Jahrb. **34**:489–520. *pls. 5, 6. fig. 1.* 1905.

[23] GIBSON, R. J. HARVEY, The axillary scales of aquatic monocotyledons. Jour. Linn. Soc. Bot. **37**:228–237. *pls. 5, 6.* 1905.

Aponogetonaceae, Juncaginaceae, Alismaceae, Butomaceae, and Hydrocharidaceae. From an investigation of adult structure and manner of development, he has concluded that the axillary scales found at the bases of the leaves in the plants of these genera are homologous with the more specialized and solitary stipules of Selaginella and Isoetes. It will be recalled that GIBSON regards the ligule as a sort of specialized ramentum, protecting and keeping moist the young leaves and growing apex of Selaginella and Isoetes.—FLORENCE LYON.

Reserve food of trees.—NIKLEWSKI[24] confirms by macrochemical methods the observation of RUSSOW and of FISCHER, that in winter the fat-content of trees first increases and then diminishes. The process cannot be reversed by temperature changes. While a rise of temperature accelerates the formation of fat, no change affects its solution. The transformation of fat and of starch are not related. Low temperatures promote the formation of sugar from starch. Complex phenomena result from a rise of temperature. So great is the loss of reserves by the increased respiration, that it seems probable that bodies other than starch or fat share in the metabolism and give rise to carbohydrates.—C. R. B.

Conjugation of yeasts.—GUILLIERMOND[25] has extended his studies on the conjugation of yeasts to several additional forms of the Schizosaccharomyces and Zygosaccharomyces. The union of the cells is followed by the fusion of the two nuclei, after which the fusion nucleus divides and the two cells separate or spores are formed in the fusion cell. In some forms conjugation takes place with the germination of the spores. GUILLIERMOND regards this cell and nuclear fusion as a sexual act, but of course chiefly on physiological grounds. Since we do not know the history of the yeasts, it is a matter of speculation whether or not these conjugating cells are phylogenetically gametes.—B. M. DAVIS.

Amphispores in Uredineae.—ARTHUR has given an account of all species of rusts which have amphispores,[26] *i. e.*, as defined by CARLETON, one-celled spores which resemble the teleutospores of Uromyces in appearance, but have two or more germ-pores, and in germination behave like uredospores, their function seeming to be to tide the fungus over unfavorable conditions. This account includes one species of Uromyces and eight of Puccinia, one of which, *P. Garrettii*, is new. All the forms are American, for thus far no cases of the occurrence of amphispores have been reported from other parts of the world.—H. HASSELBRING.

Photosynthesis extra vitam.—BERNARD has again examined carefully the

[24] NIKLEWSKI, B., Untersuchungen über die Umwandlung einiger stickstoffreier Reservestoffe während der Winterperiode der Bäume. Beihefte Bot. Centralbl. **19**[1]: 68–117. 1905.

[25] GUILLIERMOND, M. A., Recherches sur la germination des spores et la conjugaison chez les lévures. Rev. Gén. Bot. **17**:337–376. *pls. 6–9. figs. 11.* 1905.

[26] ARTHUR, J. C., Amphispores of the grass and sedge rusts. Bull. Torr. Bot. Club **32**:35–42. *figs. 9.* 1905.

question of photosynthesis *in vitro*, and again with negative results.[27] He repeated MACCHIATI'S experiments (following his directions *in litt.*), and tried also those of MOLISCH, which lent faint support to MACCHIATI'S conclusions. The gas disengaged seems due only to bacterial infection and when obtained at all does not conform in amount to that demanded by theory. This accumulation of negative results makes exceedingly doubtful the claims of FRIEDEL and MACCHIATI.—C. R. B.

Measuring transpiration.— CANNON describes[28] a method of studying the rate of transpiration upon plants in place, which he calls the polymeter method, because LAMBRECHT'S portable polymeter, a combined hygrometer and thermometer is used to ascertain the increase in humidity of the atmosphere around the experimental plant when enclosed in a bell jar. Certain defects in the method are noted, but the most important one, that it itself produces a variable decrease in transpiration, is not mentioned.— C. R. B.

Diastase.— KLEEMANN, finding the known methods of determining the course of diastase formation not sufficiently accurate, proposes a new, and, as he claims, more satisfactory one.[29] Using it he has determined that the amount of diastase formed depends, on the one hand, upon the water content of the barley, and on the other, upon how the water is supplied and taken up, and that the loss by respiration is greater the greater the water content.— C. R. B.

The sporophyte of mosses.—TRUE finds[30] that the nodding of the capsusel of Mnium, and probably of Funaria also, is due to geotropic stimulation, while the direction of illumination determines the plane of the curve in the seta, the apex of the capsule sometimes curving toward and sometimes away from the incident light. The calyptra affords important protection to the growing sporophyte from mechanical injury and desiccation.—C. R. B.

Chloroform a stimulant.— So Miss Latham[31] finds it in small quantities to Sterigmatocystis, especially at the time of germination, while larger quantities are inimical or fatal. Less acid formation and less sugar consumption under the stimulus indicate greater metabolic economy.—C. R. B.

Chromosome reduction.—A useful collective review of the recent literature on this subject is presented by KÖRNICKE in Bot. Zeit. **63**[2]: 289–307. 1905.—C. R. B.

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[27] BERNARD, C., Sûr l'assimilation chlorophyllienne. Beihefte Bot. Centralbl. **19**[1]: 59–67. 1905.

[28] CANNON, W. A., A new method of measuring the transpiration of plants in place. Bull. Torr. Bot. Club **32**: 515–529. 1905.

[29] KLEEMANN, A., Untersuchungen über Malzdiastase. Landw. Versuchsstat. **63**: 93–134. 1905.

[30] TRUE, R. H., Notes on the physiology of the sporophyte of Funaria and Mnium. Beihefte Bot. Centralbl. **19**[1]: 34–44. 1905.

[31] LATHAM, M. E., Stimulation of Sterigmatocystis by chloroform. Bull. Torr. Bot. Club **32**: 337–357. 1905.

NEWS.

DR. ENRICO PANTANELLI has been appointed docent in botany at Rome.

ÉMILE BOUDIER, the eminent mycologist, has been elected director of the *Association internationale de géographie botanique* for the year 1906.

PROFESSOR Dr. A. RICHTER has been appointed director of the botanic garden of the University of Kolosvár, the post recently vacated by the death of Professor V. BORBÁS.

A PORTRAIT of Mr. FRANCIS DARWIN was lately presented to the botanical department of the University of Cambridge, where he was for many years an active investigator and instructor.

PROFESSOR HUGO DE VRIES will sail for New York about April 1, to deliver an address at the bicentennial anniversary exercises in honor of BENJAMIN FRANKLIN to be held in Philadelphia April 17–20, under the auspices of the American Philosophical Society. He expects to remain in this country two or three months.

DR. D. T. MACDOUGAL has resigned his position as assistant director of the New York Botanical Garden and has been appointed director of botanical research of the Carnegie Institute. Dr. B. E. LIVINGSTON has resigned his post as physiologist in the Bureau of Soils, U. S. Department of Agriculture, and Professor FRANCIS E. LLOYD his chair in the Teachers College of Columbia University, to accept appointments as investigators on the staff of the Desert Botanical Laboratory, with Drs. CANNON and SPALDING.

AFTER thirty years' service Sir W. THISELTON-DYER retired on December 15 from the directorship of the Royal Botanic Gardens, Kew, and was succeeded by Lieutentant-Colonel D. PRAIN, formerly director of the Botanical Survey of India, and superintendent of the Royal Botanic Gardens, Calcutta. Mr. DYER will remain at Kew till March 31 next, and till that date will continue to act as botanical adviser to the secretary of state for the colonies and as technical adviser in botany to the Board of Agriculture and Fisheries, as well as to take charge of India Office work.

FROM THE *Journal* of the New York Botanical Garden we learn that Mr. R. S. WILLIAMS has returned from two years' explorations of the Luzon, Jolo, and Mindinao, three of the Philippine Islands, bringing large and important collections of herbarium and museum material, estimated at ten to twelve thousand specimens, in spite of the loss of about three months' collections by fire.

DR. J. N. ROSE with an assistant, Mr. PAINTER, spent the summer in the arid districts of central and southern Mexico, collecting cacti, of which they

secured several hundred. Special arrangements are being made to study this family thoroughly, both in living and preserved material.

Some giant bamboos in the palm-house in the past season grew 65 feet (20^{m}) in ninety-five days, an average of about 21^{cm} per day.

FROM advance sheets of the seventeenth annual report of the Missouri Botanical Garden, we learn what extraordinary burdens the SHAW bequest has been carrying these sixteen years in the way of taxes, general and special, and real estate and street improvements. This has unhappily delayed the design of Director TRELEASE for development of the Garden as a research center, making impossible the prompt execution of the plan to maintain a staff of specialists and furnish them facilities for work. If the city and state were as just as Mr. SHAW was generous they would relieve the Garden of taxes at least, since it exists solely for the public good. Notwithstanding these unexampled inroads upon its income the institution has not stood still; the garden has not only been maintained but greatly improved; a fine library and herbarium has been accumulated, and notable researches have been published annually. The grounds now embrace 65 acres, the plant houses cover 30,000 square feet, the cultivated plants number 16,000 species, noteworthy groups being the cacti (678 sp.), bromeliads (204 sp.), and orchids (942 sp.). The library is now undoubtedly the best botanical library in the United States, and the herbarium contains over half a million specimens. We congratulate the Director and Trustees on the wise administration of their trust in the face of serious difficulties and discouragements.

THE American Mycological Society held its third annual meeting in connection with the American Association for the Advancement of Science at New Orleans, January 1, 1906. In the absence of the president, CHARLES H. PECK, the vice-president, F. S. EARLE, presided. The new constitution recommended by the joint committee of the Botanical Society of America, the Society for Plant Morphology and Physiology, and the American Mycological Society, as a basis for the union of the three societies, was adopted and the present officers continued as a committee with power to cooperate in the completion of the details of reorganization. The following papers were read: J. C. ARTHUR, Some reasons for desiring a better classification of the Uredinales; S. M. TRACY, Uredineae of the Gulf States; W. G. FARLOW, Some peculiar fungi new to America; F. S. EARLE, North American gill fungi; BRUCE FINK (by title), Lichens and recent conceptions of species; E. M. FREEMAN, The affinities of the fungus of *Lolium temulentum;* C. L. SHEAR, *Peridermium cerebrum* Peck, and *Cronartium Quercuum* (Berkeley); C. L. SHEAR, Romularia: An illustration of the present practice in mycological nomenclature; P H. ROLFS, Notes on cultures of Collelotrichum and Gloeosporium; P. SPAULDING, The occurrence of *Fusoma parasiticum* Tubeuf in this country; P. H. ROLFS, Notes on *Pachyma cocos;* P. H. ROLFS, *Penicillium glaucum* on pineapple fruit.—C. L. SHEAR.